ANTIAGGRESSIVE ACTIVITY OF *NYCTANTHES ARBOR- TRISTIS* LEAVES IN RODENTS

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Summary

Hydroalcoholic extract of leaves of *Nyctanthes arbor-tristis* Linn (NATE) was pharmacologically validated for its antiagressive properties in experimental animals using various models such as Foot shock-induced aggression, Isolation-induced aggression, Resident-intruder aggression and Water competition test .Extract was given orally at two different dose levels (250 and 500 mg/kg) once daily for three consecutive days, while Diazepam (2.5mg/kg), was administered as positive control .NATE. (250 and 500mg/kg) on all the models produced significant antiagressive effects such as a reduction in vocalizations along with lowering of leaping, running, rearing and facing each other, increased latency time to first attack, reduced spout gaining frequency along with a reduction in time spent which was found to be statistically significant compared to the control .Results suggested that NATE showed significant antiagressive activity.

Keywords: Antistress, *Nyctanthes arbor-tristis*,

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Introduction

Anxiety is a feeling of apprehension, nervousness, or fear. The source of this uneasiness is not always known or recognized. An exaggerated or fearful response to an appropriate or inappropriate condition may be observed during anxiety (1). Increased anxiety provokes elevated aggression. Such exaggerated responses may induce subtle alterations of different integrated systems resulting in undesirable symptoms of emotional reactivity reflected as aggression. Therefore aggression is an "overt behavior with the intension of inflicting physical damage on the opponent"(2).Aggression generally ensues due to conflicting interests associated with restricted territory, electrical, sensory, chemical stimulation or with the removal of positive re-enforcements (3). Although aggression is an adaptive response, if it is prolonged it can have serious repercussions on the health and social behavior of the individual. Numerous natural remedies have found acceptance as anxiolytic agents as they diffuse the unwanted effects produced by synthetic agents.

Nyctanthes arbor-tristis, (Fam.Oleaceae) is commonly known as Parijatham, Harsinghar and Night Jasmine. The leaves, flowers, seeds and bark of *Nyctanthes arbor-tristis* are widely used in traditional remedies and folkloric medicines in India. Widely distributed throughout India and also cultivated in gardens for its fragrant flowers (4, 5). The fresh juice obtained from the leaves of the plant found to have anti malarial activity (6). The 50% ethanolic extract of the seeds, leaves, roots, flowers and stem of the plant has been proved to posses antiamoebic (7) and anti allergic properties (8). Leaf extract of the plant showed anti-inflammatory (9), analgesic, antipyretic and ulcerogenic activities (10). Immunostimulant activity of the leaves, seeds and flowers of the plant has been reported (11). The water soluble fraction of the ethanolic extract has been proved to posses tranquilizing, antihistaminic, purgative effects (12) and depletion of tumor necrosis factor - α (13).The arbortristoside A isolated from the seeds found to have antitumor activity (14).Many iridoid glycosides have been isolated from the leaves and seeds of the plant. These include arborside A, arborside B and arborside C (15). The triterpenoides lupeol, oleanolic acid, friedelin and nyctanthic acid were isolated from the leaves of the plant (16) . Naringenin-4'-0-β-glucopyrenosyl-α-xylopyranoside (17) has been isolated from the fresh stems of the plant. β-monogentiobioside of α - crocin or crocin 3, β-monogentiobioside-β-D-monoglucoside ester of α-crocin or crocin 2 and β-digentiobioside ester of α-crocin or crocin1 (18) have been isolated from the corolla tubes of flowers of *Nyctanthes arbor tristis* . Earlier, we have earlier reported the anxiolytic activity (19) and antidepressant activity (20) of leaf extract of *Nyctanthes arbor- tristis*. The present study is designed to evaluate antiaggressive activity of leaf extract of *Nyctanthes arbor tristis* in experimentally induced aggression in rats.

Material and Method

Preparation of plant extracts

The leaves of *Nyctanthes arbor-tristis* were collected from the local garden of Lucknow, India in May 2007. The plant material was identified and authenticated taxonomically at National Botanical Research Institute, Lucknow. A voucher specimen (LWG accessions No. 94392) of the collected sample was deposited in the institutional herbarium for future reference. The powdered leaves of *Nyctanthes arbor-tristis* (5 kg) were passed through S.S.sieve (20mesh) before extraction. Plant material was successively extracted with ethanol (50%) in soxhlet apparatus. The crude extract obtained was concentrated in a rotary evaporator under reduced pressure and freeze dried to yield 15.6 %w/w (NATE).

Animals

Adult albino rats (150-180g) and Wister mice (25-35g) of either sex were obtained form the Animal House of the Institute and were randomly distributed into different experimental groups. The rats were housed in groups of six in polypropylene cages at an ambient temperature of 25±10C and 45-55% RH with a 12:12 h light /dark cycle. Animals were provided with commercial food pellets and water ad libitum. All studies were performed in accordance with the guide for the care and use of laboratory animals.

Drug treatment

In the acute toxicity study no deaths were observed during the period at the doses tested up to 2000 mg/kg. Hence, the NAT was administered orally at two different dose levels (250 and 500 mg/kg) once daily for three consecutive days. Control group of animals received suspension of 1% CMC in distilled water. Diazepam (2.5 mg/kg, i.p.) was used as standard drug and were administered intraperitoneally to rodents 30 min. before experiments for comparison. Experiments were conducted on day 3, one hour after the last drug administration.

Safety evaluation

NATE was administered to 10 mice and 10 rats in a dose of 2g/Kg p.o and observations were made for gross behavioral changes such as locomotion, rearing, respiration, tremors, passivity, righting reflex, lacrimation and mortality for 14 days (21).

Assessment of anti aggressive activity

The four most widely used rodent models were chosen to evaluate the effect of *NATE* on aggressive behavior, *viz.*: foot shock-induced aggression, isolation-induced aggression, resident intruder aggression and water competition test.

Foot shock-induced aggression

Weight matched Swiss mice were divided into four groups (each containing 6 pairs), treated with vehicle, *NATE* (250, and 500 mg/kg BW) or diazepam respectively, once daily for 14 consecutive days. On the 14th day, 1 h after the last oral treatment, all pairs of mice were subjected to foot shock by placing them in an aggressometer (Techno) for 3 min. During a 3 min observation period, every 5 sec a 60-Hz current was delivered for 5 sec. Each pair of mice was dosed and tested without previous exposure. The total number of fights were recorded for each pair (2*2, 23*).

Isolation-induced aggression

Male Swiss mice (body weight of 25 ± 5 g) were kept isolated in small cages for two months. Prior to the drug treatment, the aggressive behavior of the isolated mouse was assessed against a male mouse (similar in weight to that of the isolated mouse, and accustomed to living in a group) and put into the cage of an isolated mouse for 5 min. Immediately, the isolated mouse started to attack the "intruder". The aggressive behavior of the isolated mouse was characterized by hitting the tail on the bottom of the cage, screaming and biting. Isolated mice not exhibiting aggressive behavior were excluded from the test. One day after the initial trial, isolated animals were distributed into four groups (n = 6) and were treated with vehicle, *NAT*E (250, and 500 mg/kg BW) or Diazepam for 14 consecutive days. One hour after the last dose, aggressive behavior of the isolated mouse against a male mouse was evaluated for 5 min (*23-25)*. Aggressive behavior related parameters assessed during this test were latency to first attack, screaming, pursuit frequency, tail rattle, aggressive posture, and total number of fights.

Resident-intruder aggression

Male rats (400 ± 20 g) were tested in their home cages for aggression against a smaller (200 ± 20 g) male intruder. Before the start of the experiments, each resident male rat was kept in a pair with one female rat in a polypropylene cage for 15 days, and they were randomly divided into four groups (n = 6). Drug treatment was started from the 16th day onward, and only male rats of each pair were administered with vehicle, *NATE* (250, and 500 mg/kg BW) or diazepam for 14 consecutive days. The resident female was removed from the cage 30 min prior to the start of the test. One hour after the last oral treatment, a male intruder (~ 200 g) was placed in the territorial cage of the resident male, and behavior of the resident male was observed for the next 15 min. During this period, the time until the first attack (in seconds), number of attacks, and duration of each attack (in seconds) were recorded by a blind observer (23).

Water competition test

Albino rats of Wister strain (250-400g) were paired and housed in different cages. After 6 days of acclimatization, animals were deprived of water for 23 hrs. At the end of this, pairs of water deprived animals were administered with the vehicle and 60 minutes later, a water bottle having a spout was introduced such that only one animal of a pair can drink at a time. Frequency and time in seconds of spout possession were recorded for 5 minutes
and the aggressive animal of the pairs was marked for identification. Animals were then provided with water for 55 minutes after which the water bottle was withdrawn for 23 hours. The test was repeated on the second day. On day-2 instead of the control drug, the aggressive animals received Diazepam (2.5mg/kg), 250mg/kg and 500mg/kg *of NATE* which was administered 60 minutes prior to the recording and aggressive bursts were observed for 5 minutes (26).

Statistical analysis

The values were represented as mean ± S.E.M. for six rats. Analysis of variance (ANOVA) test was followed by individual comparison by Newman–Keuls test using Prism Pad software for the determination of level of significance.

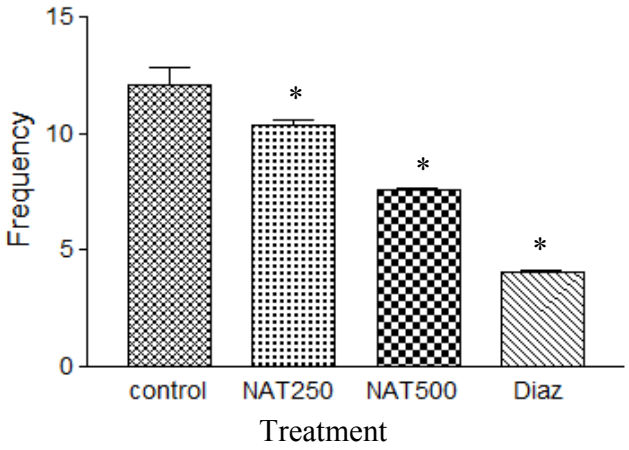
Results and Discussion

No untoward observations such as gross behavioral changes and mortality were seen for 14 days of observations.

Foot shock-induced aggression

NATE extract significantly reduced the total number of fight as compared to control (Figure 1).

Figure 1. Effect of *Nyctanthes arbor - tristis* on foot shock-induced aggressive behavior.

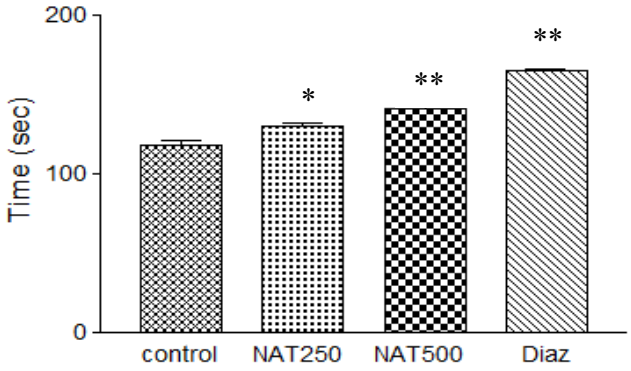


Control = 6 & treatment extract n=6, *P< 0.001 compared to control

Isolation-induced aggression

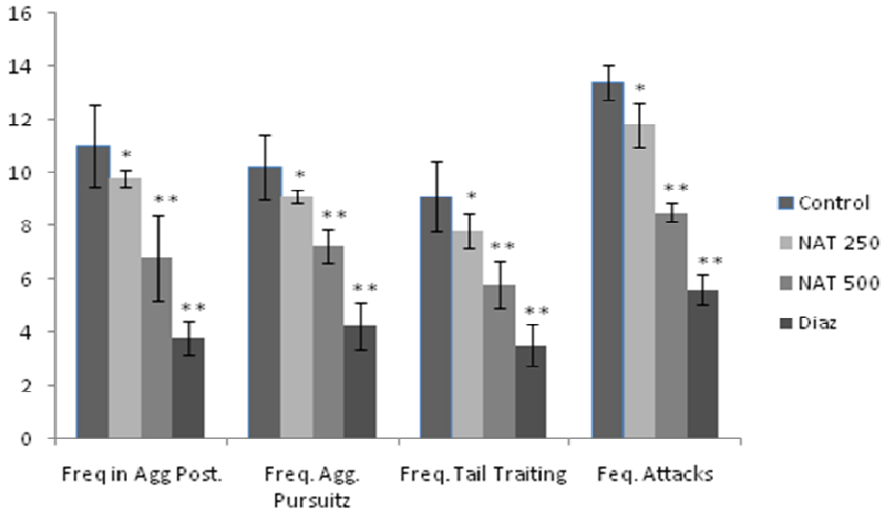
Both the doses of *NATE* (250, and 500 mg/kg) significantly increased latency time to first attack (Figure 2) while the number of aggressive postures, aggressive pursuit, tail traiting
frequency and attacks were significantly reduced by all two doses of *NATE*. These effects of *NATE* (250, and 500 mg/kg) were identical to that of diazepam (2.5 mg/kg) (Figure 3).

Figure 2. Effect of *Nyctanthes arbor- tristis* on latency time to isolation-induced first attack.



Control = 6 & treatment extract n=6, *P 001 compared to control

Figure 3. Effect of *Nyctanthes arbor- tristis* on frequency of various isolation-induced aggressive behavior.

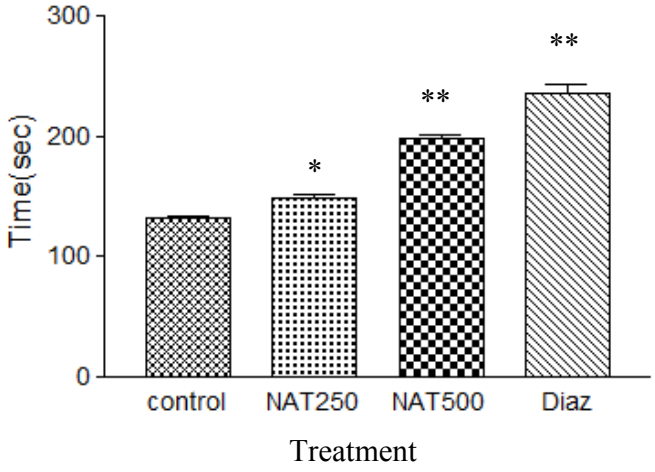


Control = 6 & treatment extract n=6, * $p < 0.05$, ** $p < 0.001$ compared to control

Resident-intruder aggression

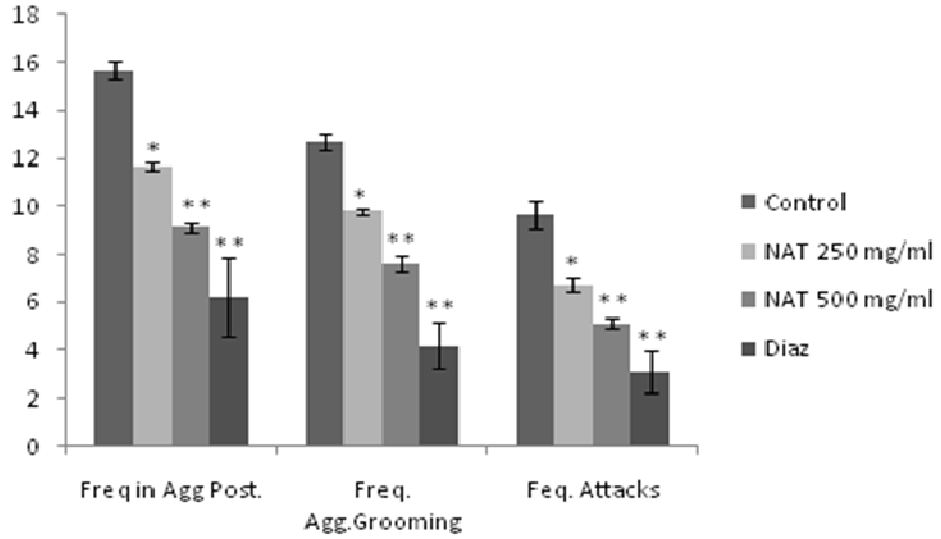
Both the doses of NATE (250, and 500 mg/kg) significantly prolonged the latency period of first attack (Figure 4) and significantly reduced the frequency of aggressive posture, aggressive grooming and total number of attacks (Figure 5). The total duration of fighting was also reduced significantly by both the doses (250 and 500 mg/kg) of NATE (Figure 6). The observed effects of diazepam in this model were qualitatively similar to those of NATE.

Figure 4. Effect of *Nyctanthes arbor- tristis* on latency period of first attack against resident intruder.



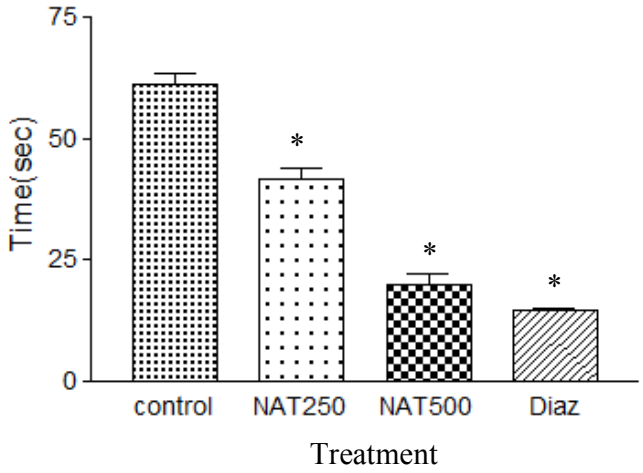
Control = 6 & treatment extract n=6,*P< .05, **P<0.001 compared to control.

Figure 5. Effect of *Nyctanthes arbor- tristis* on frequency of various resident intruder aggressive behaviors.



Control = 6 & treatment extract n=6,*P< .01, **P<0.001 compared to control

Figure 6. Effect of *Nyctanthes arbor- tristis* on total duration of fighting against resident intruder.

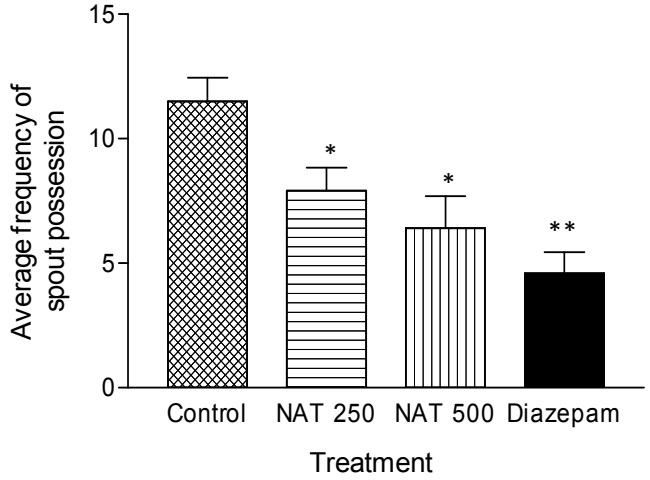


Control = 6 & treatment extract n=6, *P< .0.001 compared to control.

Water competition test

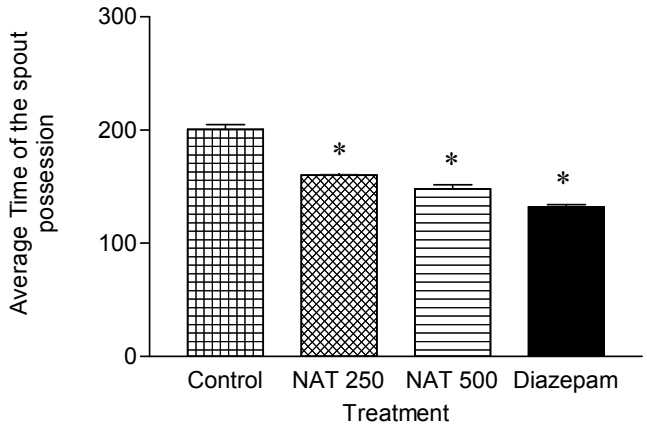
The average time of spout possession and frequency of spout possession in the water competition test are depicted in fig.7 and fig.8. *NATE* at a dose of 200mg/kg and 500mg/kg produced a significant reduction in spout gaining frequency along with a reduction in time spent which was found to be statistically significant ($p<0.05$) compared to the control.

Figure 7. Effect of *Nyctanthes arbor- tristis* on average frequency of spout possession.



Control = 6 & treatment extract n=6, *P< .0.001 & *P< .0.005 compared to control.

Figure 8. Effect of *Nyctanthes arbor- tristis* on average time of the spout possession.



Control = 6 & treatment extract n=6, *P< .0.001 as compared to control.

One of the difficult medical and social problems associated with neuropsychiatric sturbances is aggression. Analysis of aggression in different animal species could provide a firm understanding of human violence and the therapeutic measures to be taken to combat it (27). Exposure of an animal to a threatening situation elicits a behavioral repertoire referred to as aggression. The behavioral profile may be manifested in the form of offensive and defensive aggression (28). Offensive behavior is characterized by initiative of the aggressor and devastation to the opponent (29, 30). On the other hand defensive behavior lacks initiative and the animal does not impose intentional damage (30). In animal aggression, the involvement of different neuronal circuits has been demonstrated. The initiation and execution of aggression is related to the disturbance in the fine balance of serotonin, dopamine, gamma amino butyric acid (GABA) and their

particular receptor subtypes. Aggressive laboratory animals generally display reduced brain 5-HT turnover (31). The term aggression is widely employed to indicate various patterns of psychological or sociological behavior resulting from pathological, biochemical or physiological alteration of central nervous system constituents. There are many psychiatric disorders such as schizophrenia and Alzheimer's disease which show close association with aggression (3*2*). Like any other behavior, aggression is also controlled and modulated by neurotransmitters. The agonist of 5-HT1A/5-HT1B and antagonist of 5-HT2A/5- HT2C receptors have been reported to possess antiaggressive properties (3*3, 34*). Bernard *et al*. (3*5*) showed that dopamine levels and measurement of dopamine synthesis and turnover in the whole brain have increased in aggressive strains of mice and in mice that have just engaged in aggressive behavior. In the isolation-induced aggressive behavior model the level of dopamine increases in the striatum (3*6*). Tsuda *et al*. (37) and Tanaka *et al*. have reported that expression of aggression is an alternative mechanism to decrease the stress related increase of nor adrenaline.

The present anti-aggressive study was carried out to explore knowledge about the beneficial effect of *NATE* in the parameters of aggression. The paradigm such as foot shock induced aggression and water competition test provide an assessment of the rodents level of defensive behavior, which in turn would reflect the extent of anxiousness or fear the animal experiences (26). In rats, defensive behavior is composed of a host of responses like vocalization, leaping, rearing, running and facing each other which depend on the nature of threat the animal envisages. In the water competition test, water deprivation induces high rank motivation resulting in competition for a common goal which is "water" Therefore it serves to demonstrate that the animal with greater dominance or aggression will take hold of the spout faster and for a longer duration. If the drug administered minimizes this urge it would indicate the anxiolytic property of the drug. The placidity in behavior induced in animals by *NATE* may be due to its ability to alter the level of neurotransmitters and Na+/K+-ATPase. The enzyme Na+/K+-adenosine triphosphatase (Na+/K+-ATPase) has an accomplished role in regulating brain function, such as repolarization of the neuronal membranes and neurotransmitters uptake/release. Individual variations in the activity of brain Na+/K+ATPase may result in differences in the functioning of the brain, which, in turn, could produce behavioral divergences (38). Stimulation of the Ca+2 receptor could induce the release of 5- hydroxytryptamine (39). The enhanced turnover of 5-HT can cause blunting of aggression and could be a plausible reason for the antiaggressive property of *Nyctanthes arbor-tristis*. In this regard the effect of *NATE* on serotonin levels is of particular interest.Antidepressants, anxiolytics, cognitive (4*0)* function modulators, anticonvulsants, and other psychoactive agents are now identified as potential anti-aggressive therapeutics because of their neurotransmitter modulator properties. NATE has been investigated in various experimental models of depression (20), anxiety (19) and memory and learning to reveal its modulator action on a variety of neurotransmitters.

In conclusion, the total aqueous extract of *Nyctanthes arbor-tristis* was found to be efficacious in producing serenity and masking the constellation of behavioral changes encountered during aggressive bouts making it a promising naturally derived product.

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References

1. Blanchard DC, Griebel G and Blanchard RJ., Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. Neurosci. Biobehav Rev. 2001; 25: 205-218.
2. Moyer KE. Kinds of aggression and their physiological basis. Common. Behav Biol. 1968; 2A: 65-87.
3. Katherine Simpson. The role of testosterone in aggression. M J M. 2001; 6: 32-40. December 17-20, 2006
4. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed., Dehradun, India: Oriental Enterprises, 1935; pp. 131-134.
5. Singh K.L, Roy R, Srivastava V, Tadon JS, Mishra R. Aarborside D, a minor iridoid glucoside from *Nyctanthes arbor-tristis* . J Nat Prod 1995; 58:1562- 1564.
6. Badam L, Rao TLG, Wagh UV. Antimicrobial activity of fresh leaf' juice of *Nyctanthes arbor-tristis* Linn. in vitro. Indian J Parasitol, 1987; 11: 13-14.
7. Chitravanshi VC, Singh AP, Ghoshal S, Prasad K, Srivastava V, Tandon JS. Therapeutic action of *Nyctanthes arbor-tristis* against Caecal amoebiasis of rat. Int J Pharmacog 1992; 30: 71-75.
8. Gupta PP, Srimal RC, Srivastava M, Singh KL, Tandon AS. Antiallergic activity of arbortristosides from *Nyctanthes arbor-tristis*. Int J Pharmacog .1995; 33: 70-72.
9. Saxena, R.S., Gupta, B., Saxena, KK, Singh RC, Prasad RC. Study of anti-inflammatory activity in the leaves of *Nyctanthes arbor- tristis* linn.- an Indian medicinal plant. J thnopharmacol 1984; 11: 319-330.
10. Saxena RS, Gupta B, Saxena KK, Srivastava VK, Prasad DN. Analgesic, antipyretic and ulcerogenic activity of *Nyctanthes arbor- tristis* leaf extract. J Ethnopharmacol 1987; 19: 193-200.
11. Puri A, Saxena R, Saxena RP, Saxena KC, Srivastav AV,. Tandon JS. (1994) Immunostimulant activity of *Nyctanthes arbor-tristis*. J Ethnopharmacol 1994; 42: 31-37
12. Saxena RS, Gupta B, Lata S. Tranquillizing, antihistaminic and Purgative activity of *Nyctanthes arbor-tristis* leaf extract. J Ethnopharmacol 2002; 81: 321-325.
13. Paul BN, Saxena AK. Depletion of tumor necrosis factor-alpha in mice from *Nycthanthes arbor-tristis*. J Ethnopharmacol 1997; 56: 153-158.
14. Susan T, Muzaffer A, Purushothaman KK. Inhibitory activity of arbortristoside A on fibrosarcoma in albino rats. Arogya 1986; 12: 122-130.
15. Srivastava V, Rathore A, Ali SM, Tandon JS. New benzoic esters of loganin and 6-beta-hydroxy loganin from *Nyctanthes arbor-tristis* J Nat Prod 1990; 53:303-308.
16. Rimpler H, Junghanns JU. Nyctanthosid, ein neues iridoid aus *Nyctanthes arbor-tristis*. Tetrahedron Lett 1975; 30: 2423.
17. Chauhan JS, Saraswat M. A new· glycoside from the Stem of *Nyctanrhes arbor-tristis*. J Indian Chem Soc 1978; 55: 1049-1051.
18. Dhingra VK, Seshadri TR, Mukherjee SK. Carotenoid glycosides of Nyctanthes arbor-tristis. Indian J Chem 1976; 14B: 231.
19. Tripathi S, Tripathi PK, Vijaya kumar M, Rao Ch.V, Singh PN. Anxiolytic Activity of Leaf Extract of *Nyctanthes arbor-tristis* In Experimental Rats.Pharmacologyonline 2010; 2: 186-193
20. Tripathi S, Tripathi PK, Singh PN. Antidepressant Activity of of *Nyctanthes arbor-tristis* Leaf Extract Pharmacologyonline 2010; 3: 415-422

21. Ghosh MN. Fundamentals of experimental pharmacology. 2nd ed., Scientific Book Agency, Calcutta, 1984; p.156.
22. Jain K, Barar FSK. Central cholinergic involvement in Clonidine and shock-induced aggression, and its modification by nitrazepam, haloperidol and propranolol: An experimental study in albino mice. Indian J Pharmacol. 1985; 17:34-41.
23. Vogel HG (ed.). Drug Discovery and Evaluation: Pharmacological Assays. 2nd ed., Springer-Verlag, Heidelberg, Germany, 2002; pp. 425-430.
24. Plummer HK, Holt I. Effect of alprazolam and triazolam on isolation-induced aggression in rats. Ohio J Sci. 1987; 87:107-111
25. Muehlenkamp F, Luciont A, Vogel WH. Effects of selective serotonergic agonists on aggressive behavior in rats. Pharmacol Biochem Behav. 1995; 50:671-674.
26. Gerhard Vogel H. Drug discovery and Evaluation.2nd ed., Springer, 2002; p.428.
27. Klaus A, Miczek, Eric Fish W, Joseph de Bold F and Rosa de Almeida MM.Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine and gaminobutyric acid systems. Psychopharmacology. 2002; 163: 434-458
28. Adams DB. Brain mechanisms for offense, defense and submission. Behav. Brain Sci. 1979; 2: 201-224.
29. Blanchard RJ, Blanchard DC and Takahashi T.Attack and defensive behavior in the albino rat. Anim.Behav.1977; 25: 622-34.
30. Oliver B, Van Dalem D and Hartog J. A new class of psychoactive drug serenics. Drugs Future.1990; 11: 473-499
31. Nelson RJ and Chiavegatto S. Molecular basis of aggression. Trends Neurosci. 2001; 24: 713-719.
32. Hollander E, Tracy KA, Swann AC, Coccaro EF, McElroy SL, Wozniak P, Sommerville KW, Nemeroff CB. Divalproex in the treatment of impulsive aggression: Efficacy in cluster B personality disorders.Neuropsychopharmacology. 2003; 28:1186-1197.
33. Sijbesma H, Schipper J, de Kloet ER, Mos J, Van Aken H, Olivier B. Postsynaptic 5-HT1 receptors and offensive aggression in rats: A combined behavioral and autoradiographic study with eltoprazine. Pharmacol Biochem Behav. 1991; 38:447-458.
34. Mos J, Olivier B, Poth M, Van Aken H. The effects of intraventricular administration of eltoprazine, 1-(3-trifluoromethylphenyl) piperazine hydrochloride and 8-hydroxy-2-(di-n-propylamino) tetralin on resident intruder aggression in the rat. Eur J Pharmacol. 1992; 212:295-298.
35. Bernard BK, Finkelstein ER, Everett GM. Alterations in mouse aggressive behavior and brain monoamine dynamics as a function of age. Physiol Behav. 1975; 15:731-736
36. Tizabi Y, Thoa NB, Maengwyn-Davies GD, Kopin IJ, Jacobowitz DM. Behavioral correlation of catecholamine concentration and turnover in discrete brain areas of three strains of mice. Brain Res. 1979; 166:199-205.
37. Tsuda A, Tanaka M, Ida Y, Shirao I, Gondoh Y, Oguchi M, Yoshida M. Expression of aggression attenuates stress induced increases in rat brain noradrenaline turnover. Brain Res. 1988; 474:174-180.
38. Rosana Alves, José Gilberto, Barbosa de Carvalho and Marco Antonio Campana Benedito. Brain Research.2005; 1058: 178-182
39. Kuo-peing Liu, Andrew Russo F, Shu-chi Hsiung, Mella Adlersberg, Thomas Franke F, Michael Gershon D and Hadassah Tamir .Calcium receptor-Induced Serotonin Secretion by Para follicular Cells: Role of Phosphatidylinositol 3-Kinase-Dependent Signal Transduction Pathways. The Journal of Neuroscience. 2003; 23(6): 20491
40. Tanaka T, Yoshida M, Yokoo H, Tomita M, Tanaka M.Expression of aggression attenuates both stress-induced gastric ulcer formation and increases in noradrenalin release in the rat amygdala assessed by intracerebral microdialysis. Pharmacol Biochem Behav. 1998; 59:27-31.