

ATTENUATION OF STRESS-INDUCED BEHAVIORAL DEFICITS BY *AZADIRACHTA INDICA* (NEEM): ROLE OF SEROTONIN

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Abstract

The present study was designed to investigate the effect of *Azadirachta indica* (Neem) leaf extract on restraint-induced behavioral deficits and changes in serotonin metabolism in rats. Exposure to a single stress decreased food intake, growth rate and elicited anxiogenic-like behavior on an elevated plus maze. Prior administration of neem leaf extract @ 1 ml/kg for 5 days attenuated stress induced behavioral deficits of food intake, growth rate and anxiogenic behavior but the level of anxiety in unrestrained animals was not altered. Restraint-stress did not alter brain tryptophan and 5-hydroxytryptamine (5-HT) levels. 5-hydroxyindolacetic acid (5-HIAA) concentration increased in saline but not in neem injected rats. Administration of neem leaf extract increased brain tryptophan and decreased brain 5-HT concentration in unrestrained animals. The present study showed that neem extract could attenuate anxiogenic and appetite suppressant effects of stress by decreasing brain 5-HT and 5-HIAA concentration.

Introduction

Stress acts as a predisposing and precipitating factor in the onset of affective illness specially depression (Brown *et al.*, 1987). Parallel studies on experimental animals show that an uncontrollable stressor produces neurochemical and behavioral deficits. In similar studies it has been shown that an episode of 2 hours restraint stress decreased food intake, growth rate and locomotor activity in rats (Haleem *et al.*, 1988; Samad *et al.*, 2002). On repeated immobilization these behavioral deficits were no longer observed (Haleem & Parveen, 1994). These studies suggested that behavioral adaptation to a stress schedule develops when the same stress is administered repeatedly. A variety of stress stimuli increase the synthesis and turnover of 5-hydroxytryptamine (5-HT, serotonin) in the whole brain and various brain regions of rats (Haleem *et al.*, 1988). On the other hand, when an episode of 2 hours restraint stress challenged to rats which were adapted repeatedly restrained schedule, 5-HT synthesis did not increase (Haleem & Parveen, 1994).

Azadirachta indica (Neem) is an evergreen tree in the Indian subcontinent. Leaves and seeds have been used in Ayurvedic medicine. Previous studies showed that neem leaves could reduce anxiety and stress when ingested in small quantities (Banerjee, 1992). The present study was designed to monitor the effect of neem leaves extract on restraint-stress induced behavioral deficits. Associated changes of brain serotonin metabolism were also determined.

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Materials and Methods

Animals and treatment: Locally bred Albino Wistar rats weighing 200 to 220 g were used for the experiment. They were caged individually in plastic cages with free access to cubes of standard rodents diet and tap water for 5 days, before starting the experiment.

Preparation and dosage of neem extract: Ten g of fresh neem leaves were grinded in 100 ml distilled water and liquid squeezed was centrifuged. The supernatant was injected intraperitoneally (IP) to rats @ 1 ml/kg body weight. Control animals were injected with saline (0.9% NaCl: 1 ml/kg body weight).

Experiment protocol: The animals were divided into saline injected and neem injected groups. These animals were injected with neem extract and saline daily for 6 days. On the 4th day open field activity was monitored, after one hour of injection of neem extract or saline. On the 5th day, animals of the two groups were subdivided into restrained and unrestrained groups. One hour after the injection, a group of neem injected animals and another group of saline injected animals were restrained on wire grids for 2 hours. The other group of neem injected and saline injected animals were left unrestrained in their home cages. Cumulative food intake and body weight changes for 24 hours were monitored on the 6th day. Activity on an elevated plus maze were also monitored on the 6th day after the neem extract or saline injection. The animals were decapitated on the 6th day to collect brain samples 2 hours after the neem extract or saline injection. Brain samples were stored at -70°C for the estimation of tryptophan, 5-HT and 5-HIAA by HPLC-EC (Haleem & Parveen, 1994).

Restraint stress: The animals were restrained on wire grids of 10"x9" fitted with a Perspex plate of 9"x6.5". Restraining procedure was same as described earlier (Haleem & Parveen, 1994). The animals were restrained between 11:00 a.m. to 1:00 p.m. After 2 hours of restraining period the animals were released and returned to their home cages.

Open field activity: The open field apparatus used in the present investigation consisted of a square area 76x76 cm with walls 42 cm high. The floor was divided by lines into 25 equal squares. To determine the activity, a rat was placed in the centre square of the open field and latency to leave the centre square and number of squares crossed with all four paws were scored for 5 minutes as described earlier (Haleem, 1996). The activity of saline injected rats and neem injected rats were monitored in a balanced design to avoid order effect on the 4th day between 11:00 a.m. to 2 p.m.

Plus maze activity: The plus maze apparatus used in the present investigation consisted of four arms in which two were open and two were closed. The arms were of identical length (50 cm) and width (10 cm). Arms were joined by central area of 5 cm. The maze was elevated from the floor at a height of 60 cm. To determine the activity, a rat was placed in the centre of plus maze and time spent in the open and closed arms, number of entries in open and closed arm were determined (Samad *et al.*, 2002). The activities of saline injected restrained and unrestrained, and neem injected restrained and unrestrained rats were monitored for 5 min., in a balanced design on the 6th day between 9:00 a.m. to 12:00 a.m.

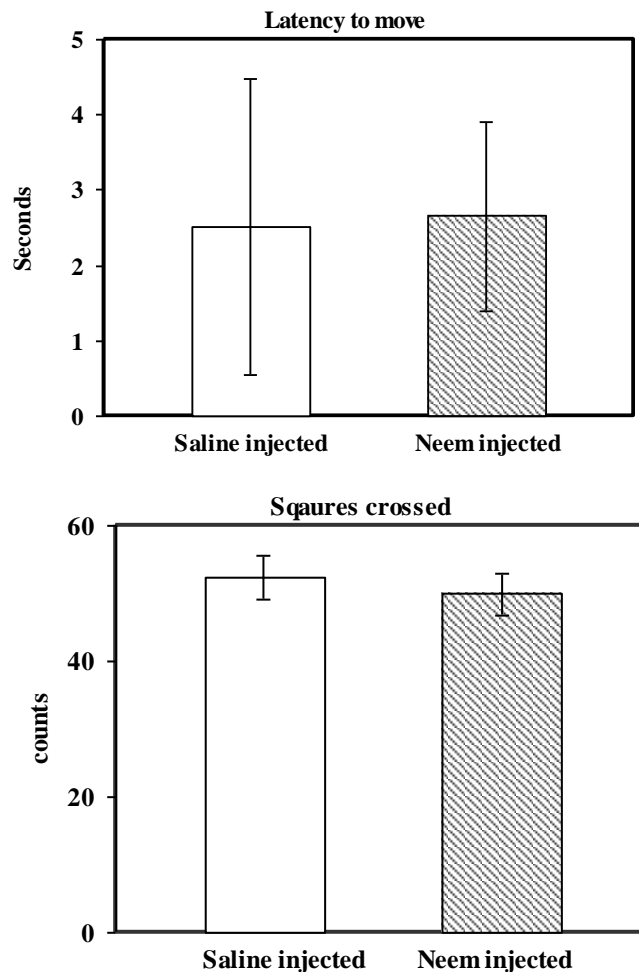


Fig. 1. Effect of repeated administration of neem leaves extract on open field activity. Values are means \pm S.D. ($n=12$). Differences by t-test were not significant.

Statistical analysis: Data on open field activity were analyzed by Student's t-test. Data on the effects of 2 hours restraint stress on behavior and on brain tryptophan and 5-HT metabolism were analyzed by two-way ANOVA. Post-hoc comparisons were done by Newman-Keuls statistic. P-values <0.05 were taken as significant.

Results

The effect of 4 days saline or neem extract injection on open field activity in rats is shown in Fig. 1. No significant effect was observed on latency to move and squares crossed.

The effect of 2 hours restraint stress on 24 h cumulative food intake and growth rate in rats treated with saline and neem leaf extract is shown in Fig. 2. A significant effect of

stress was observed on food intake ($F=289.04$ $df=1,20$ $p<0.01$) and on growth rate ($F=76.51$ $df=1,20$ $p<0.01$). Effect of neem injection on food intake ($F=8.76$ $df=1,20$ $p<0.01$) and on growth rate ($F=19.27$ $df=1,20$ $p<0.01$) were also significant. Interaction between stress and neem injection was not significant for food intake ($F=0.77$ $df=1,20$ $p>0.05$) and significant for growth rate ($F=4.70$ $df=1,20$ $p<0.05$). Post-hoc analysis showed that 2 h restraint stress decreased 24 h cumulative food intake and growth rate in saline as well as neem injected rats. The decreases were smaller in neem injected rats. Food intake and growth rates of unrestrained animals were highly comparable.

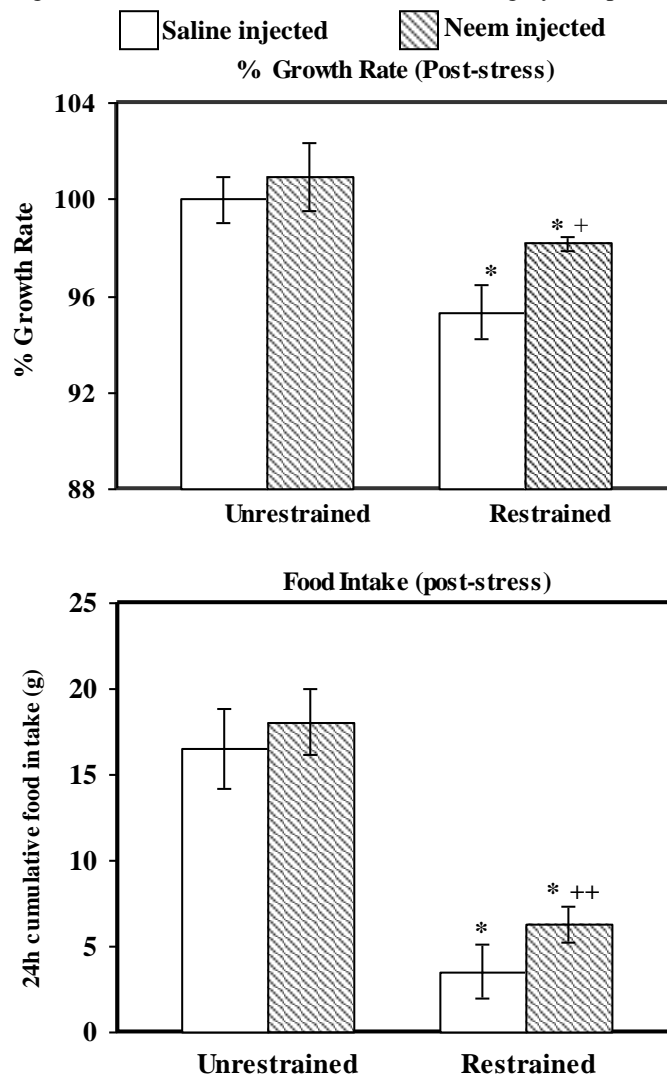


Fig. 2. Effect of 2 hours restraint stress on 24 h cumulative food intake and growth rate in animals treated with saline and neem leaves extract. Values are means \pm S.D. ($n=6$). Significant differences by Newman-Keuls test: * $p<0.01$ from respective unrestrained animals, + $p<0.05$, ++ $p<0.01$ from saline treated restrained animals following two-way ANOVA.

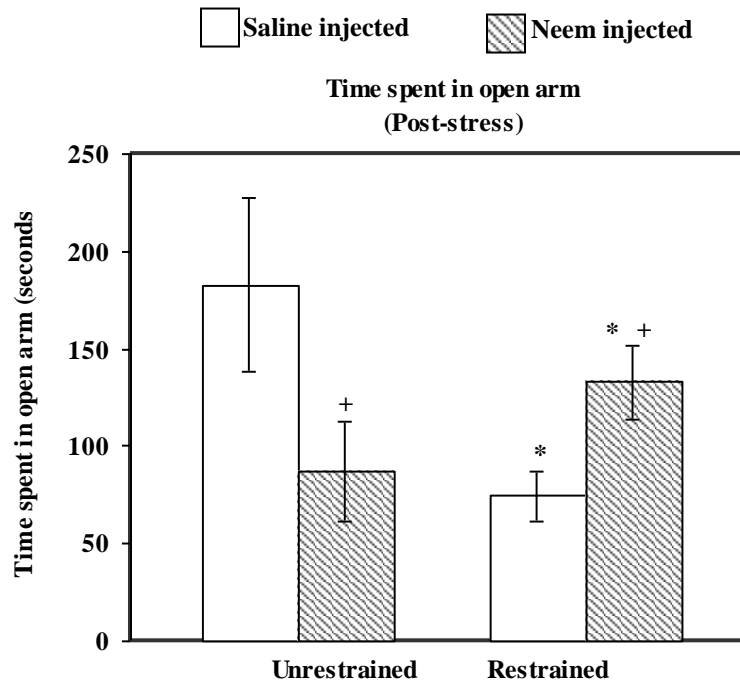


Fig. 3. Effect of 2 h restraint stress on plus maze activity in saline and neem injected rats. Values are means \pm S.D. (n=6). Significant differences by Newman-Keuls test: * $p < 0.01$ from respective unrestrained animals, + $p < 0.01$ from saline treated animals following two-way ANOVA.

The effect of 2 hours restraint stress on plus maze activity in saline and neem injected animals is shown in Fig. 3. A significant effect of stress ($F=7.19$ $df=1,20$ $p < 0.05$) and a non-significant effect of neem injection ($F=2.64$ $df=1,20$ $p > 0.05$) was observed. Interaction between stress and neem injection ($F=45.11$ $df=1,20$ $p < 0.01$) was also not significant. Post-hoc analysis revealed that 2 hours restraint-stress decreased open arm ambulatory activity in repeatedly saline injected rats. The deficits were smaller ($p < 0.01$) in repeatedly neem extract injected rats.

The effect of 2 hours restraint-stress on brain tryptophan, 5-HT and 5-HIAA levels of saline and neem injected animals is shown in Fig. 4. Data on brain tryptophan showed non-significant effect of stress ($F=2.94$ $df=1,20$ $p > 0.05$), significant effect of neem injection ($F=26.57$ $df=1,20$ $p < 0.05$) and non-significant interaction ($F=0.40$ $df=1,20$ $p > 0.05$) between the stress and neem injection. Post-hoc analysis showed that 2 hours restraint stress did not alter brain tryptophane levels in saline or neem injected rats. Administration of neem extract for 6 days significantly increased tryptophan levels in unrestrained as well as restrained rats.

Data on 5-HT levels revealed a significant effect of stress ($F=13.9$ $df=1,20$ $p < 0.01$) and significant effect of neem injection ($F=13.92$ $df=1,20$ $p < 0.01$). A non-significant interaction ($F=1.46$ $df=1,20$ $p > 0.05$) was observed between the stress and neem injection. Post-hoc analysis showed that 2 hours restraint stress decreased ($p < 0.01$) 5-HT levels in saline but not in neem extract injected rats. Repeated administration of neem extract decreased ($p < 0.01$) 5-HT levels in unrestrained animals.

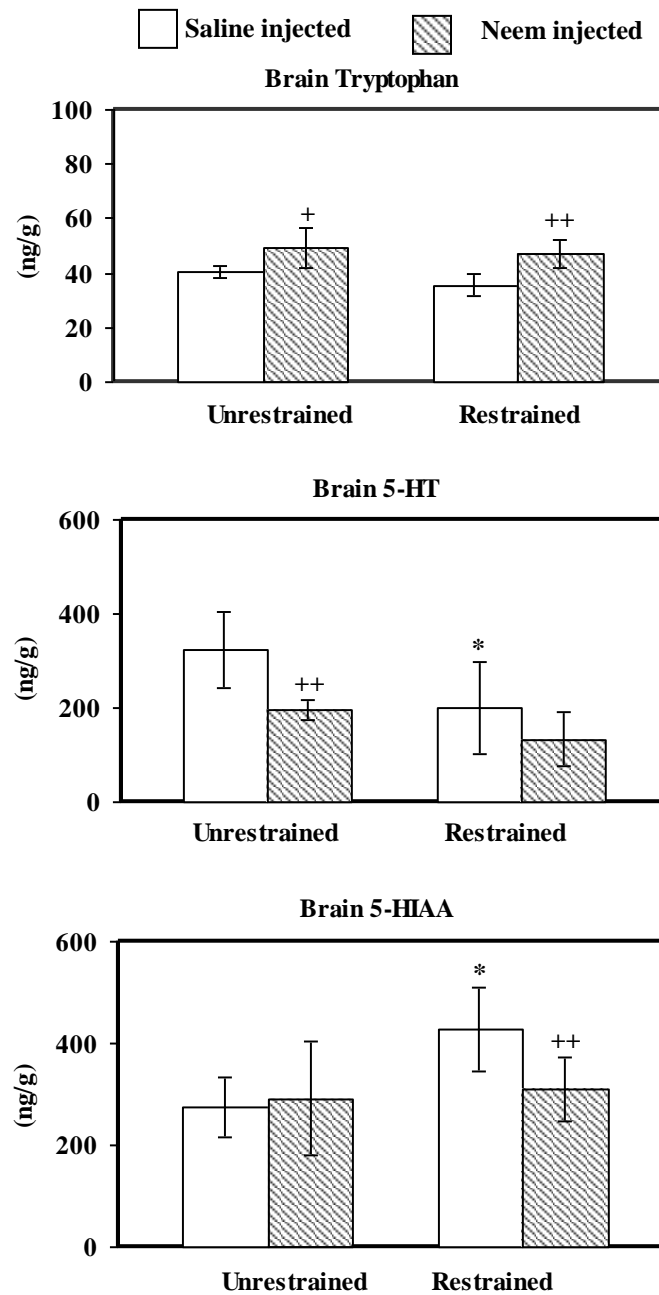


Fig. 4. Effect of 2 hours restraint stress on whole brain tryptophan, 5-HT and 5-HIAA concentration in neem and saline injected rats. Values are means \pm S.D. (n=6). Significant differences by Newman -Keuls test: *p<0.01 from respective unrestrained animals, +p<0.05, ++p<0.01 from saline treated animals following two-way ANOVA.

Data on 5-HIAA levels revealed significant effect of stress ($F=14.73$ $df=1,20$ $p<0.01$), neem injection ($F=5.02$ $df=1,20$ $p<0.01$) and a significant interaction ($F=9.13$ $df=1,20$ $p<0.01$) between the stress and neem injection. Post-hoc analysis showed that 2 hours restraint stress increased ($p<0.01$) 5-HIAA levels in saline injected but not in neem injected rats. A significant decrease was observed following 2 hours restraint stress in neem extract injected rats.

Discussion

Rats exposed to an episode of immobilization stress for 2 hours exhibited a decrease in 24 hours cumulative food intake and growth rate (Haleem *et al.*, 1988; Samad *et al.*, 2002). Locomotor activity monitored 24 hours later also decreased (Kennett *et al.*, 1988; Haleem & Parveen, 1994). In the present study, when saline treated rats were restrained, food intake and body weights were significantly decreased, which is consistent with the previous study (Haleem *et al.*, 2002). An additional finding of the present study is that prior administration of neem leaf extract for 5 days before exposing rats to restraint stress attenuated restraint-induced decrease of food intakes and growth rates.

There are reports that immobilization stress decreased exploratory activity of rats in the open arm of an elevated plus maze (Guimardes *et al.*, 1993; Padovan *et al.*, 1993; Tacko Hata, 2001). In the present study we also found that 2 hours restraint stress decreased the exploration of open arm in saline treated animals. Neem leaf extract attenuated the restraint-induced deficits of elevated plus maze exploration. The results suggested an anxiolytic profile of neem leaf extract.

There are reports that stress causes activation of sympathetic nervous system which is responsible for stress-induced increase in brain tryptophan (Dunn *et al.*, 1991) and serotonin levels (Haleem & Parveen, 1994). An important finding of the present study was that repeated administration of neem leaf extract increased brain tryptophan levels in both unrestrained and restrained animals. An increase in brain serotonin concentration, reported previously in neem treated rats (Jaiswal *et al.*, 1994) was not observed in the present study. Restraint-induced increase in 5-HT turnover reported previously (Haleem & Parveen, 1994) was also observed in the present study in saline injected animals, was not observed in neem leaf extract treated animals. On the other hand, neem leaf extract injected restrained animals exhibited a significant decrease in 5-HIAA concentration than saline injected restrained animals. It would suggest that extract of neem leaves could attenuate stress-induced anxiety and deficits of food intake and the effects are possibly mediated by a decrease in 5-HT metabolism by neem.

References

- Banerjee, S. and H. Rembold. 1992. *Azadirachtin* A interfere with control of serotonin pools in the neuroendocrine system of locusts. *Naturwissenschaften*, 79: 81-84.
- Brown, G.W., A. Bifulco and T.O. Harris. 1987. Life events vulnerability and onset of depression some refinements. *Br. J. Psychiat.*, 150: 30-42.
- Dunn, A.J. and J. Welch. 1991. Stress-induced and endotoxin-induced increases in brain tryptophan and serotonin metabolism depend on sympathetic nervous system activity. *J. Neurochem.*, 57: 1615-1622.
- Guimardes, F.S., E.A. Del Bel, C.M. Padovan, S. Mendoca Netto and R. Titze-de-Almeida. 1993. Hippocampal 5-HT receptors and consolidation of stressful memories. *Behav. Brain. Res.*, 58: 133-139.
- Haleem, D.J., G.A. Kennett and G. Curzon. 1988. Adaptation of female rats to stress: shift to male pattern by inhibition of corticosterone synthesis. *Brain Res.*, 484: 339-47.

- Haleem, D.J. and T. Parveen. 1994. Effect of restraint on rat brain regional 5-HT synthesis rate following adaptation to repeated restraint. *Neuro Report*, 5: 1785-88.
- Haleem, D.J., Z.S. Saify, F. Batool and M.A. Haleem. 2002. Pre and postsynaptic receptors to 1-(1-nephthyl piperazine) following adaptation to stress in rats. *Prog. Neuropharmacol. Biol. Psychiatry*, 26: 149-56.
- Hata, T., H. Nishokawa, E. Itoh and Y. Funakami. 2000. Anxiety-like behavior in elevated plus maze test in repeatedly cold-stressed mice. *Jpn. J. Pharmacol.*, 85: 189-196.
- Jaiswal, A.K., S.K. Bhattacharya and S.B. Acharya. 1994. Anxiolytic activity of *Azadirachta indica* leaf extract in rats. *Indian J. Exp. Biol.*, 32: 489-91.
- Kennett, G.A. and G. Curzon. 1988. Evidence that hypophagia induced by m-CPP and TEMPP requires 5-HT 1C and 5-HT 1B receptors; hypophagia induced by RU24969 only requires 5-HT 1B receptors. *Psychopharmacol.*, 96: 93-100.
- Samad, N., T. Parveen, S. Haider and D.J. Haleem. 2002. Effect of repeated injection of neem leaf extract on a model of anxiety and brain serotonin metabolism in rats. *J. Hamdard Medicus.*, 45: 54-58.

(Received for publication 28 April 2005)