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Effect of dichloromethane fraction of *Areca catechu* nut on monoamines associated behaviors and tyramine pressor sensitivity in rodents

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Abstract: The current study was aimed at investigating the effect of *Areca catechu* nut dichloromethane fraction (7 mg/kg) on monoamines (serotonin and dopamine) modulation (5-hydroxytryptophan-induced tremors and phenylethylamine-induced stereotypes) and its interaction with tyramine (cheese effect). The dichloromethane fraction caused pronounced increase in 5-HTP-induced tremors (50%) with negligible PEA-induced stereotypes (20%). Additionally, it did not produce a significant increase in the tyramine pressor effects. These results suggest that the dichloromethane fraction of *A. catechu* nut primarily elevates serotonin levels (probably *via* monoamine oxidase A inhibition) and does not induce cheese effect.

Keywords: *Areca catechu* nut; dichloromethane fraction; serotonin; dopamine; cheese effect.

INTRODUCTION

The *Areca catechu* nut is a famous chewing nut intended for mastication in various parts of the globe, especially the Indo-Pak subcontinent. An estimated 600 million persons consume it daily (Nelson and Heischober, 1999) making it the 4th most commonly used psychoactive substance after caffeine, nicotine and alcohol (Gupta and Ray, 2004). It has been used as anthelmintic, visceral infections, diarrhea, dysentery, burns, ulcers, dentrifice, stimulant, exhilarant, digestive, astringent, cardiac and nervine tonic in past (Raghavan and Baruah, 1958). It has also been reported to inhibit MAO-A activity (Dar and Khatoon, 1997) and elevate 5-HT and NA levels (Abbas *et al*., 2012) in rats favoring anti-depressant-like properties. *A. catechu* alkaloids have been the focus of investigation, particularly arecoline which has been considered as an active constituent underlying most of the biological activities elicited by nut (Nelson and Heischober, 1999).

Monoamine oxidase inhibitors (MAOIs) are clinically effective in the treatment of depressive disorders with a better therapeutic potential outcome against atypical depression (Liebowitz *et al*., 1988). Additionally, some patients with typical endogenous depression who are refractory to tri-cyclic antidepressants (TCAs) may respond favorably to MAOIs (Freeman *et al*., 1993). However, their use is restricted because of adverse effects particularly cheese effects (Anderson *et al*., 1993) induced by tyramine-rich diet (e.g. cheese and red wine) which is contraindicated in the patients undergoing non-selective monoamine oxidase inhibitors treatment. Tyramine, a sympathomimetic amine induces release of stored monoamines (such as noradrenaline and dopamine) metabolized by the enzyme monoamine oxidase and if co-administered with MAOIs may cause hypertensive crisis and trigger cluster headaches (McCabe, 1986). Therefore, patient counseling and appropriate diet planning for patients on MAOIs is critical to avoid such drug interactions. Because of their selectivity towards MAO-A, a new generation MAOIs (e.g. moclobemide) appeared to be safer thereby, leaving MAO-B intact to deaminate tyramine (Keller *et al*., 1987, Plenker *et al*., 1997). They are well tolerated and have low liability for potentiating the tyramine pressor effect (Dowson, 1987) and are as effective as some TCAs such as chloroimipramine (Civeira *et al*., 1990).

The current study was aimed at investigating the effect of dichloromethane fraction of *A. catechu* nut on serotonin and dopamine modulation in rats. The possibility of a cheese effect was also explored using tyramine pressor test. Phenelzine (irreversible and nonselective MAOI) and moclobemide (reversible and selective MAO-A inhibitor) were included as standard drugs.

MATERIALS AND METHODS

Chemicals

Phenelzine sulfate, phenylethylamine, L-5-hydroxy-tryptophan and tyramine hydrochloride were purchased from Sigma USA. Moclobemide was a gift from Roche, Pakistan.

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Animals

Either sex of Sprague Dawley rats (200-300g) were provided by the animal house of Aga Khan University, Karachi (Total number =120). They were kept under standard condition (light/dark cycle of 12 h each and free access to food and water). All experiments were performed in accordance with the guidelines provided by the National Institute of Health (NIH publication no. 85-23, revised 1985). for the care and use of laboratory animals.

Preparation of dichloromethane fraction

A.catechu nuts were purchased from a local market and authenticated by a botanist at the University of Karachi. A voucher specimen (No. 67278) was submitted to the herbarium of the Department of Botany, University of Karachi. The dichloromethane fraction was obtained as described earlier (Dar and Khatoon, 2000).

L-5-HTP-induced tremors

Animals were intraperitoneally treated (n=10/group) with saline (control group) or dichloromethane fraction of *A. catechu* (7 mg/kg) or moclobemide (5 mg/kg) or phenelzine (16 mg/kg). After 1 h of treatment, each animal received injection of L-5-HTP (100 mg/kg, *i.p.*). After 30 min, the generalized tremors were noted for 1 h (Lessin, 1959).

PEA-induced sterotypes

Animals were intraperitoneally treated (n=10/group) with saline (control group) or dichloromethane fraction of *A. catechu* (7 mg/kg) or moclobemide (5 mg/kg) or phenelzine (16 mg/kg). After 1 h of treatment /administration, each animal received injection of PEA (25 mg/kg, *i.p.*). After 15 min, animals were observed for the presence of stereotyped behavior (mouth movements and licking) for 1h (Braestrup *et al*., 1975).

Tyramine pressor test

The animals were anaesthetized (pentothal sodium, 70 mg/kg) and the trachea was intubated to facilitate spontaneous respiration. The right carotid artery and left jugular vein were cannulated and blood pressure was recorded as described previously by (Dar *et al*., 1999). The effect of tyramine (0.1, 0.3 and 0.5 mg/kg, n=5/group) on blood pressure, heart rate and the duration of its pressor effect were determined. After each dose of tyramine, noradrenaline (0.5 μM/kg) was administered to replenish its stores with an interval of 10 min.

The dose of tyramine that produced a rise of 30 mm Hg in blood pressure was selected to study its interaction with dichloromethane fraction, moclobemide and phenelzine as described by (Caille *et al*., 1996). The IC_{50} values of above mentioned compounds obtained from our earlier antidepressant studies were used (Dar and Khatoon, 2000). Animals were intraperitoneally treated (n=5/group) with saline (control) or dichloromethane fraction of *A. catechu* (7 mg/kg) or moclobemide (5 mg/kg) or phenelzine (16 mg/kg). After 1 h of treatment animals were anaesthetized for blood pressure recording as described before and tyramine (0.3 mg/kg) was administered. The percent increase in blood pressure (amplitude), heart rate and the duration of pressor effect was noted and compared with their respective control.

STATISTICAL ANALYSIS

The data was presented as mean ± S.E.M. Difference between various means were computed by one-way ANOVA using SPSS. In L-5-HTP-induced tremors and PEA-induced sterotypes, n is 10 per group. In tyramine pressor test, n is 5 per group.

RESULTS

Effect of A. catechu nut dichloromethane fraction, moclobemide and phenelzine on potentiation of

a) L-5-HTP-induced tremor in rats

The L-5-HTP (100 mg/kg) did not induce tremors in rats. However, in the presence of phenelzine (16 mg/kg), moclobemide (5 mg/kg) or dichloromethane fraction (7 mg/kg) a 30%, 70% and 50% increase in tremors, respectively was evident (table 1).

Table 1: Effect of *A. catechu* nut dichloromethane fraction of, moclobemide and phenelzine on potentiation of L-5-HTP-induce tremors in rats.

Treatment (mg/kg)	Percent of tremors
Dichloromethane fraction (7 mg/kg) + L-5-HTP (100 mg/kg)	50
Moclobemide (5 mg/kg) + L-5-HTP (100 mg/kg)	70
Phenelzine (16 mg/kg) + L-5-HTP (100 mg/kg)	30
Saline + L-5-HTP (100 mg/kg)	00

Number of animals per group (control and test) = 10.

b) PEA-induced stereotypes in rats

The PEA (25 mg/kg) did not induce stereotype behavior in rats. However, in the presence of phenelzine (16 mg/kg) and dichloromethane fraction (7 mg/kg), there was 80% and 20% increase in behaviors, whereas moclobemide (5 mg/kg) did not induce any behavioural changes (table 2).

Effect of tyramine on blood pressure, heart rate and duration of pressor effect in anesthetized rats

Intravenous administration of tyramine (0.1-0.5 mg/kg) caused dose dependent increase in blood pressure, heart rate and duration of pressor effect. At 0.5 mg/kg, significantly maximum increase of 39±3.1% and 15±2.9% was observed in blood pressure and heart rate of animals, respectively, that was reversed to baseline after almost 7 min (table 3).

Table 2: Effect of dichloromethane fraction of *A. catechu* nut, moclobemide and phenelzine on potentiation of PEA-induce stereotypes in rats

Treatment (mg/kg)	Percent of stereotypes
Dichloromethane fraction 7 mg/kg + PEA 25 mg/kg	20
Moclobemide 5 mg/kg + PEA 25 mg/kg	00
Phenelzine 16 mg/kg + PEA 25 mg/kg	80
Saline + PEA 25 mg/kg	00

Number of animals per group (control and test) = 10.

Effect of A. catechu nut, moclobemide and phenelzine on blood pressure and duration in tyramine pressor test

Tyramine (0.3 mg/kg) caused an increase in blood pressure (22±2.1%) that was reversed to baseline after 2 min without affecting the heart rate (table 4). Pretreatment of animals with phenelzine induced a marked potentiation in amplitude (45±0.5%) and duration (7.4±0.8%) of the pressor effect of tyramine. Although the amplitude and duration of tyramine pressor effect was also increased by the pretreatment of moclobemide but increase in amplitude (1.4x) and duration (2.7x) was less compared to that of phenelzine. However, the pretreatment of animals with dichloromethane fraction of *A. catechu* did not cause a significant elevation in either amplitude or duration of tyramine pressor effect.

DISCUSSION

The current study was aimed to investigate the effect of *A. catechu* nut (dichloromethane fraction) on monoamines (serotonin and dopamine) associated behaviors in rats. The possibility of cheese effect was also explored using tyramine pressor test.

The L-5-hydroxytryptophan is an amino acid and precursor of monoamine neurotransmitter serotonin and its elevated levels lead to a serotonin syndrome characterized by symptoms such as tremors (Sallinen *et al*., 1998). Our data showed that L-5-HTP (100 mg/kg) alone did not induce tremors in rats (table-1). However, in the presence of the dichloromethane fraction (7 mg/kg) a 50% increase in the tremors were noticeable indicating that the effect of L-5-HTP has been potentiated. In other words, the fraction has elevated the levels of serotonin in rat brain. This is in agreement with our recent report (Abbas *et al*., 2012) where serotonin levels in rat hippocampus were raised following areca nut treatment. Additionally, phenelzine (non-selective MAO inhibitor) and moclobemide (selective MAO-A inhibitor) induced 30% and 70% increase in tremors, respectively suggesting that MAO-A inhibitors has greater ability to potentiate L-5-HTP effects most likely due MAO-A inhibition which is mainly responsible for degradation of serotonin. This also led us to deduce that *A. catechu* nut (dichloromethane fraction) is most likely a MAO-A inhibitor as reported earlier (Dar and Khatoon, 2000).

Phenylethylamine is a naturally occurring substance in the brain which elicits stereotype behavior (SB, repetitive movements of head, neck and mouth) due to the release of catecholamines, mainly dopamine (Naik *et al*., 1978). PEA (25 mg/kg) did not induce SB in rats (table-2), however, in the presence of dichloromethane fraction, a 20% increase in SB indicates that the PEA effect has mildly potentiated levels of dopamine in the rat brain. This is in agreement with the lack of a significant alteration in hippocampal dopamine levels following areca nut treatment (Abbas *et al*., 2012). Additionally, the phenelzine 16 mg/kg (non-selective MAO inhibitor,) induced 80% increase in SB and moclobemide 5 mg/kg (selective MAO-A inhibitor,) 0%, respectively. This suggests that MAO-B inhibitors have the potential of enhancing the effect of PEA probably because MAO-B is mainly responsible for degradation of dopamine. This also led us to deduce that *A. catechu* nut (dichloromethane fraction) is probably not an inhibitor MAO-B enzyme.

Tyramine (present in diet such as cheese), metabolized by MAO, causes release of stored monoamines (noradrenaline and dopamine) leading to increase in blood pressure and heart rate (Iwata *et al*., 1997). Tyramine in the presence of MAOIs (e.g. phenelzine) can lead to adverse effect like cheese effect (hypertensive crisis). Our data showed that tyramine (0.1-0.5 mg/kg) showed dose dependent increase in blood pressure, heart rate and duration of pressor effect (table-4). Pretreatment of animals with phenelzine induced a marked potentiation in amplitude (45±0.5%) and duration (7.4±0.8%) of the pressor effect induced by tyramine. Although the amplitude and duration of tyramine pressor effect was also increased by the pretreatment of moclobemide, this increase in amplitude (1.4 times) and duration (2.7 times) was less as compared to that of phenelzine. These results are in accordance with those reported by previous investigators (Caille *et al*., 1996). However, the pretreatment of animals with dichloromethane fraction of *A. catechu* nut did not cause a significant elevation in either amplitude or duration of tyramine pressor effects. Likewise, BW-130U87 and RS-8359 which are potent, reversible and selective inhibitors of rat and human brain MAO-A have been known (Cooper *et al*., 1992, Iwata *et al*., 1997). It is established that old generation MAOIs (e.g. phenelzine) inhibit both MAO-A and B, thus allowing a greater amount of tyramine to reach at the sympathetic nerve endings and provoke a serious hypertensive crisis. Whereas, the new reversible and selective MAO-A inhibitors are safer, due to their specificity in inhibiting only MAO-A (leaving the MAO-B intact to deaminate tyramine). Thereby, minimizing the

Table 3: Effect of tyramine on percent increase of blood pressure, heart rate and the duration of pressor effect in anesthetized rats

Dose (mg/kg)	Blood pressure (mm Hg)	Heart rate (beats/min)	Duration of pressor effect (min)
0.1	$12 \pm 5.3^{n.s}$	$1 \pm 0.8^{n.s}$	$0.9 \pm 0.77^{n.s.}$
0.3	$20 \pm 3.8^{**}$	$11 \pm 4.9^{n.s.}$	$3.3 \pm 1.7^{n.s.}$
0.5	$39 \pm 3.1^{***}$	$15 \pm 2.9^{***}$	$6.9 \pm 1.3^{**}$

Blood pressure (control) =118±6.9 mm Hg and heart rate 325 ± 12.6 beats per min. Values are means ± SEM of control animals (n=5) and animals per dose (n=5). Asterisks indicate significant change ($P<0.01^{**}$ and $P<0.005^{***}$) as compared to control and n.s. represents non-significant differences.

Table 4: Effect of tyramine in the absence and presence of dichloromethane fraction of A. catechu nut, moclobemide and phenelzine on blood pressure and the duration pressor effect.

Treatment	Percent increase in blood pressure (mm Hg)	Duration of pressor effect (min)
Tyramine (0.3 mg/kg)	22 + 2.1	2.1 + 0.1
Dichloromethane fraction + tyramine (0.3 mg/kg)	$25 + 2.1^{n.s}$	$2.2 + 0.2^{n.s.}$
Moclobemide (5 mg/kg) + tyramine (0.3 mg/kg)	$33 + 3.3^{*}$	$2.6 + 0.2^{**}$
Phenelzine (16 mg/kg) + tyramine (0.3 mg/kg)	$45 + 0.5^{***}$	$7.4 + 0.8^{***}$

Blood pressure (control) =126±2.9 mm Hg (n=25). Values are mean ± SEM. Asterisks indicate significant change (P<0.05*, P<0.01** and P<0.005***) as compared to control (tyramine 0.3 mg/kg) and n.s represents non-significant differences.

risk of their side effects, particularly the cheese effect (Bieck and Antonin, 1989, Fitton *et al*., 1992).

In conclusion, the dichloromethane fraction of *A. catechu* nut possesses/contains monoamines (mainly serotonin) with modulating potential without producing a detectable cheese effect similar to that of moclobemide (MAO-A inhibitor). Thus, the dichloromethane fraction has great potential as lead for antidepressants drug discovery.

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