

NEUROPHARMACOLOGICAL PROFILE OF AQUEOUS EXTRACT OF *ANAPHE VENATA* LARVA (NOTONDOTIDAE) IN RATS

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Abstract

Consumption of *Anaphe* larva had been reported to cause seasonal ataxia and impaired consciousness. Therefore this study examined the neuropharmacological and mechanism(s) of action of aqueous extract of *Anaphe venata* in rats. Behavioural effects namely rearing, stretching, sniffing and ataxia were determined after the intraperitoneal administration of aqueous extract of *Anaphe* larva in rats. Animals were divided into groups and graded doses (100, 200 and 400 mg/kg, i.p.) of extract were administered. The control group was administered normal saline (vehicle). The effects of scopolamine (3 mg/kg, i.p.), flumazenil (2 mg/kg, i.p.), naloxone (2.5 mg/kg, i.p.), and thiamine (1 mg/kg, i.p.) on the observed behavioral changes were also examined. The effects of the extract administered intraperitoneally at a dose of 200 mg/kg on the amphetamine-induced stereotypy and locomotion were evaluated. Aqueous *anaphe* extract induced significant ($p < 0.01$) stretching and ataxia behavioural effects while it inhibited rearing behaviour when compared with the vehicle-treated group. However, it had no significant effect on sniffing behaviour. Scopolamine reversed all the effects of the extract on rearing, stretching and ataxia. Both Flumazenil and naloxone only reversed the effects of the extract on stretching and ataxia-induced behaviours significantly. However, thiamine potentiated both stretching and ataxia-induced behaviours. The extract inhibited the amphetamine-induced stereotype behaviour and locomotion. In conclusion, these results showed that these *anaphe*-induced behavioural effects are mediated via cholinergic, GABAergic, opioidergic and dopaminergic receptor systems with strong muscarinic-cholinergic receptors involvement in ataxia-induced behaviour. We therefore suggest that muscarinic-cholinergic like drugs may be of benefit in the management of patients that present with clinical condition of seasonal ataxia.

Keywords: *Anaphe venata*, ataxia, chewing. cholinergic, dopaminergic, stretching, rats.

Introduction

Epidemic acute seasonal ataxic syndrome was first described among Ijesa people in Ilesa town of Western Nigeria during the rainy seasons by Wright and Morley in 1958 hence the pseudonym "*Ijesha shakes*". However, epidemics have also been reported from several other parts of Western Nigeria for example, Adamolekun and Ibikunle (1994) reported the outbreak of this seasonal acute ataxic syndrome at Ikare, south-western Nigeria. Its etiology was a subject of speculation for a long time but it was later attributed to acute thiamine hypovitaminosis resulting from the practice of entomophagy involving particular species of edible larvae of an African silk worm [*Anaphe venata* Butler (Notodontidae)] known as 'Kanni' or 'Monimoni' among these people (Adamolekun, 1992). The nutritional value and crude protein component of *Anaphe venata* has been investigated and found to contain several amino acids (Ashiru, 1988). *Anaphe venata* has also been reported to contain heat-resistant thiaminase [thiamin: base 2-methyl-4-aminopyrimidine methyl transferase] (Nishimune et al., 2000). The roasted larvae of this insect were consumed on regular basis during the raining season (July-September) along with carbohydrate meal as a protein supplement among the people of low socioeconomic class. *Anaphe venata* is an insect that is univoltine, and the annual period of wide availability of the larvae in the markets has been reported to coincide with the period of seasonal occurrence of the ataxic syndrome (Adamolekun, 1993). The disease onset is characterized by acute postprandial with a triad of cerebellar ataxia, intention tremors, and other motor abnormalities (Adamolekun et al., 1994). Previous animal studies showed that the extracts of this larva induced behavioural changes in mice (Onayade et al., 2004; Iwalewa et al., 2005). But the mechanism(s) of this phenomenon have not been explored; hence, we decided to investigate the possible mechanism(s) of *anaphe*-induced behavioural syndrome in rats.

Materials and methods

Larvae collection, identification and authentication

Dried *Anaphe venata* larvae were purchased from the market at Ile-Ife, Osun State in July, 2005 and were authenticated

by Professor W.A. Muse, Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria. The specimen was compared with an already deposited specimen at the Zoology Department, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of extracts

Powdered larvae (150 g) were extracted cold in 2 L of water with continuous shaking for 24 h in a mechanical shaker. The mixture was filtered and then freeze-dried using freeze-dryer (Freeze-dryer, model SB4, Benhay, U.K) to obtain the crude aqueous extract. The total aqueous extract obtained was 6.5 g (4.3% yield w/w). The extract obtained was dissolved in normal saline (Vehicle) to obtain a stock solution that was used in the preparation of the three doses used in this study.

Animals

Wistar albino rats of both sex, aged 8-10 weeks (150-200 g body weight) [186 rats: 93 males and 93 females] purchased from the animal house, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria were used in this study. The animals were separated by sex and housed (four to six per cage) in standard plastic cages with stainless steel coverlids and wood shavings as bedding. All rats had free access to water and were fed *ad libitum* with a commercial rodent diet (Bendel Feeds, Ewu, Nigeria). The experimental protocols were approved by the University Research Committee of Obafemi Awolowo University, Ile-Ife in accordance with the internationally accepted principles for laboratory animal use and care (EEC Directive of 1986; 86/609/EEC) (Waldegrave, 1986).

Drugs

Scopolamine hydrochloride, Naloxone hydrochloride, Flumazenil, Amphetamine sulphate, Thiamine hydrochloride (Sigma-Aldrich, St Louis, USA).

Methods

The animals were allowed to acclimatize in the laboratory for a period of one week prior to experimentation and then divided into various groups randomly (6-12 rats per group). The animals were placed directly from their home cage into an opaque plexiglas observation cage. All animals were observed and scored singly in the cage after the administration of the test substance (s). Each animal was used only once with wood shavings bedding changed after each assessment to remove olfactory cue from one animal to the other (Brown et al., 1999). The time of experiment was kept constant at 10.00 a.m - 2.00 p.m. daily (Siqueira et al., 1998). The laboratory was brightly lit, with an ambient temperature of 27 ± 2 °C. The scoring for each parameter was done over a period of 30 min for each animal.

Assessment of the effect of Anaphe extract on rearing, sniffing, stretching and ataxia

The animals were divided into four groups (n=12 per group of equal number of males and females) based on the three dose levels (100, 200, 400 mg/kg, i.p.) of the extract and control (saline: 1 ml/kg, i.p.). Behavioural activities such as rearing, sniffing, stretching and ataxia were scored in each animal following aqueous extracts of *Anaphe venata* (100, 200, 400 mg/kg, i.p.) or vehicle (normal saline) administration over a period of 30 min. Rearing is defined as lifting up of the forelimbs off the floor completely (Ajayi & Ukponmwan, 1994). Sniffing is an exploratory behavior in which the rat changes location, with rapid movement of its nose and whiskers (Barclay et al., 1982). Sniffing was assessed simply by direct observation and counting the number of sniffing. Stretching phenomenon simply involves stretching of the forelimb and hind limbs by the animal (extension or dorsal flexion of the trunk, causing lengthening of the body) (Ferrari et al., 1963; McPhie & Barr, 2000). The number of repeated episodes of stretching in 30 min was also scored. Ataxia was assessed as the number of repeated episode of falling or falling tendency upon movement (Andine et al., 1999).

Assessment of the effect of Anaphe extract and receptor antagonists on rearing, sniffing, stretching and ataxia behaviour

In another groups of rats, the effects of pretreatment with scopolamine (3 mg/kg, i.p., n=12) (Chapman et al., 1997; Eghashira et al., 2008), flumazenil (2 mg/kg, i.p., n=6-8) (Ayoka et al., 2005), naloxone (2.5 mg/kg, i.p., n=6) (Gallate et al., 1999) and thiamine (1 mg/kg, i.p., n=6) (Ciccica & Langlais, 2000) 15 min prior to extract (100, 200, 400 mg/kg, i.p.) administration respectively were also evaluated.

Assessment of the effect of aqueous extract on amphetamine-induced stereotypy and locomotion

Twelve rats were divided into two groups (Amphetamine alone and amphetamine + extract (200 mg/kg). The method of Siqueira et al. (1998) was used in determining the effect of the extract on amphetamine-induced stereotypy. Rats were allowed a minimum of 30 min to acclimatize to the observation cage prior to the experiments. The animals were pretreated with the extract (200 mg/kg, i.p.) followed by the administration of amphetamine (10 mg/kg, i.p.) (Tsai et al., 1995) 15 min later. Each of the 6

rats/group was individually observed during 30 sec every 5 min in an observation cage (45 cm x 25 cm x 25 cm) for 60 min. Stereotyped behavior was scored as follows: Complete absence of stereotyped behavior, presence of stereotyped movements of the head and intermittent sniffing (1), sniffing and chewing (2); chewing and intense licking (3). Locomotion was assessed using the method of Sturgeon *et al.* (1979) simultaneously with the stereotypy and scored as follows: stationary (0); movements within a localized area, forelimbs only (1); intermittent movements within half of the area of the cage (2); continuous movements within half of the area of the cage (3); intermittent movements within the whole area of the cage (4); continuous movements within the whole area of the cage (5).

Statistical analysis

Data were expressed as mean \pm SEM. Statistical differences were determined by the Student Newman-Keuls test (SNK) after a one-way analysis of variance (ANOVA).

Results

Effect of crude *Anaphe* extracts and receptor antagonists on rearing behaviour.

The results showed that aqueous extract of *Anaphe venata* decreased rearing behaviour in mice in a biphasic dose-dependent manner. However, pretreatment with scopolamine (3 mg/kg, i.p.) caused a significant [F(7,95)=6.154, $p < 0.0001$] reversal on this inhibitory effect. Pretreatment with flumazenil (2 mg/kg, i.p.), naloxone (2.5 mg/kg, i.p.) and thiamine (1 mg/kg, i.p.) revealed a non significant effect but each of these antagonists caused a significant [FMZ: F(7,77)=17.905, $p < 0.0001$; NAL: F(7,71)=5.882, $p < 0.0001$ and TME: F(7,71)=11.892, $p < 0.0001$] effect by increasing rearing behaviour individually (Table 1).

Effect of crude *Anaphe* extract and receptor antagonists on stretching behaviour

Stretching behaviour was increased in mice due to the administration of aqueous *anaphe* extract (100-400 mg/kg, i.p.) and pretreatment with scopolamine caused a significant [F(7,95)=2.657, $p < 0.05$] reversal of this effect while pretreatment with flumazenil, naloxone and thiamine potentiated this stretching-induced behaviour especially at the dose of 400 mg/kg [FMZ: F(7,77)=2.808, $p < 0.05$; NAL: F(7,71)=5.201, $p < 0.001$ and TME: F(7,71)=6.448, $p < 0.001$] (Table 2).

Effect of crude *Anaphe* extracts and receptor antagonists on sniffing behaviour

Sniffing behaviour was decreased dose-dependently in mice even though it was not significant, however, pretreatment with scopolamine caused a significant [F(7,95)=12.304, $p < 0.0001$] reversal of this inhibitory effect while pretreatment with flumazenil, naloxone and thiamine revealed no significant [FMZ: F(7,77)=1.750, $p = 0.1116$; NAL: F(7,71)=1.946, $p = 0.076$ and TME: F(7,71)=2.086, $p = 0.057$] effect (Table 3).

Effect of crude *Anaphe* extracts and neural modulators on ataxia behaviour

Aqueous *anaphe* extract (100-400 mg/kg, i.p.) induced significant [F(7, 95) = 9.22; $p < 0.01$] ataxia in the rat at all dose levels. This effect was completely blocked by scopolamine [F(7,95)=9.224, $p < 0.001$] at all dose levels of the crude extract used. There was significant [FMZ: F(7,77)=3.267, $p < 0.01$ and NAL: F(7,71)=4.086, $p < 0.001$] attenuation of the ataxia by flumazenil (2 mg/kg, i.p.) and naloxone (2.5 mg/kg, i.p.) at 100-200 mg/kg dose levels. However, thiamine (1 mg/kg, i.p.) significantly [F(7,71)=15.753, $p < 0.001$] potentiated ataxia dose-dependently (Table 4).

Effect of *Anaphe* extract on amphetamine-induced stereotypy and locomotion

Figure 1 showed that there were significant differences between the various treatment groups. The extract (200 mg/kg, i.p.) significantly ($p < 0.05$) inhibited amphetamine-induced stereotypy and locomotion in extract pretreated rat compared to amphetamine control.

Discussion

The neuropharmacological properties of the *Anaphe venata* larva such as the effect of the aqueous extracts on spontaneous rat behaviors (rearing, sniffing, stretching and ataxia) in a novel environment have been evaluated in this study. Rearing in animals is associated with motivational state and general arousal level (Sadile, 1995) and it is considered a central excitatory locomotor behavior (Ajayi & Ukponmwan, 1994) which may also be related to the vertical locomotor activity of rodents in a novel environment (Akanmu *et al.*, 2007). In this study it was observed that intraperitoneal administration of extracts in rats produced a decrease in vertical motor activity suggesting an inhibitory effect. Suppression of rearing by the extracts that was reversed by scopolamine, a muscarinic-cholinergic antagonist (Bentley *et al.*, 1999) therefore suggests the involvement of central muscarinic-cholinergic receptors in the mediation of the inhibitory effects of the *Anaphe* extracts. Flumazenil (a GABA antagonist),

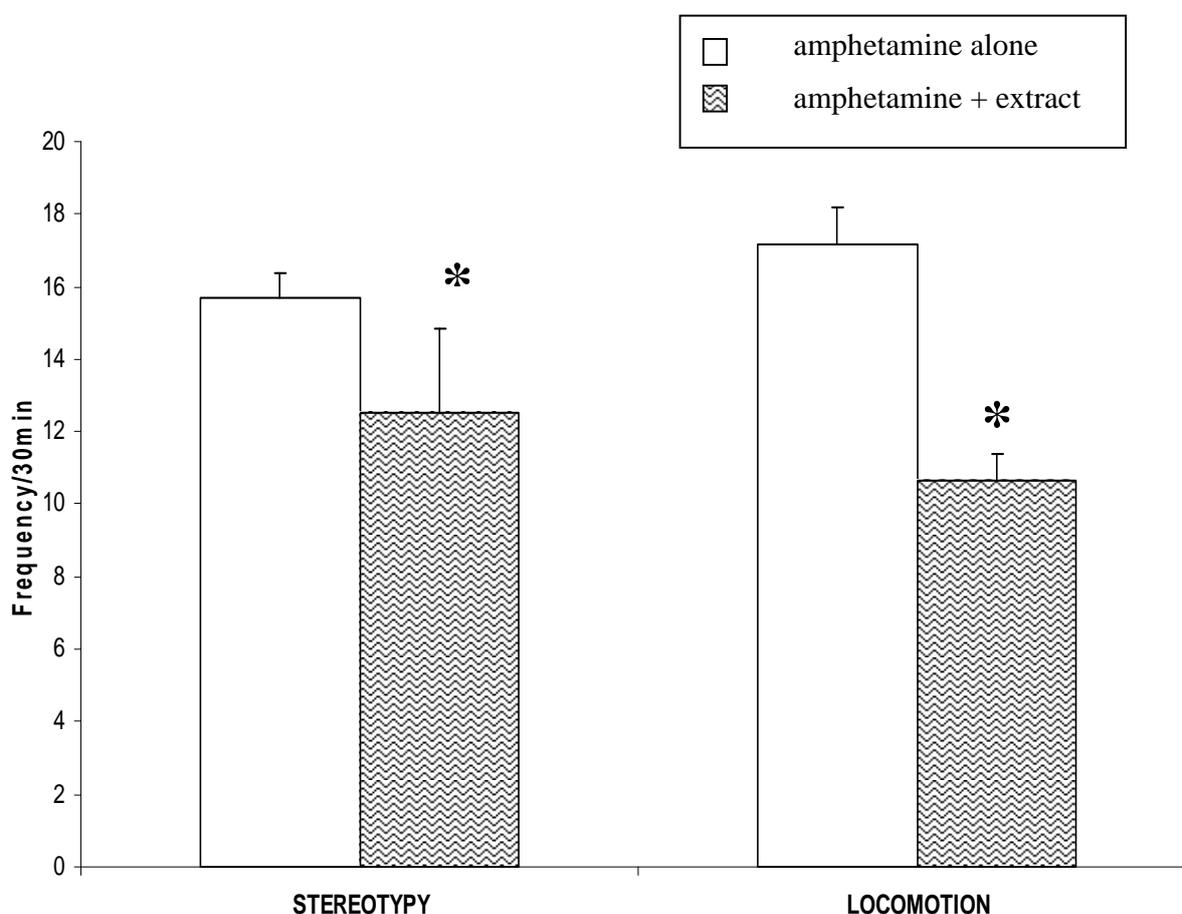


Figure 1: Effect of aqueous *Anaphe* extract on amphetamine-induced stereotypy and locomotion. Each bar is expressed as Mean \pm SEM; (n = 6) per treatment group. There were significant differences between the treatment groups compared to vehicle treated group [F (1, 11) = 6.77 p = 0.026] and F (1, 11) = 7.12, p = 0.024] for stereotypy and locomotion respectively. The extract at 200 mg/kg significantly inhibit stereotypy and locomotor behaviours respectively in extract pretreated rat (p<0.05) compared to amphetamine control group.

Table 1: Effects of aqueous extract of *Anaphe venata* on rearing behaviour in mice and the involvement of cholinergic, GABAergic, Opioidergic systems.

	Aqueous Extract of <i>Anaphe venata</i> (mg/kg, i.p.)			
	Vehicle	100	200	400
Vehicle (12)	6.83 \pm 2.54	1.67 \pm 0.53*	1.25 \pm 0.35	3.83 \pm 1.60
SCOP (n=12)	18.42 \pm 5.57*	22.75 \pm 5.02**	7.92 \pm 2.69	11.33 \pm 2.61
FMZ (n=6-8)	42.13 \pm 8.35*	8.00 \pm 2.10	5.75 \pm 1.31	7.13 \pm 2.96
NAL (n=6)	16.33 \pm 3.08*	3.33 \pm 1.05	4.67 \pm 1.27	5.33 \pm 2.35
TME (n=6)	24.00 \pm 3.84*	6.50 \pm 1.87	6.00 \pm 1.73	4.50 \pm 1.31

SCOP: Scopolamine (3 mg/kg, i.p.); FMZ: Flumazenil (2 mg/kg, i.p.); NAL: Naloxone (2.5 mg/kg, i.p.); TME:Thiamine (1 mg/kg, i.p.); *p<0.05 vs vehicle; **p<0.05 vs corresponding extract dose level.

Table 2: Effects of aqueous extract of *Anaphe venata* on stretching behaviour in mice and the involvement of cholinergic, GABAergic, opioidergic systems.

	Vehicle	Aqueous Extract of <i>Anaphe venata</i> (mg/kg, i.p.)		
		100	200	400
Vehicle (n=12)	0.00 ± 0.00	5.50 ± 2.64	5.42 ± 1.77	6.67 ± 2.08*
SCOP (n=12)	0.00 ± 0.00	2.67 ± 1.44	2.50 ± 1.24**	4.75 ± 1.23
FMZ (n=6-8)	0.00 ± 0.00	5.88 ± 2.76	13.25 ± 4.80**	5.63 ± 2.96
NAL (n=6)	0.00 ± 0.00	9.67 ± 3.07	9.67 ± 3.04	17.83 ± 3.90**
TME (n=6)	0.00 ± 0.00	14.67 ± 6.32	6.67 ± 1.77	23.17 ± 5.32**

SCOP: Scopolamine (3 mg/kg, i.p.); FMZ: Flumazenil (2 mg/kg, i.p.); NAL: Naloxone (2.5 mg/kg, i.p.); TME:Thiamine (1 mg/kg, i.p.). *p<0.05 vs vehicle; **p<0.05 vs corresponding extract dose level.

Table 3: Effects of aqueous extract of *Anaphe venata* on sniffing behaviour in mice and the involvement of cholinergic, GABAergic, opioidergic systems.

	Vehicle	Aqueous Extract of <i>Anaphe venata</i> (mg/kg, i.p.)		
		100	200	400
Vehicle (n=12)	12.33 ± 4.10	10.17 ± 1.32	9.33 ± 1.42	7.92 ± 0.83
SCOP (n=12)	106.42 ± 25.52*	50.75 ± 5.14**	52.00 ± 6.78**	30.17 ± 3.90
FMZ (n=6-8)	11.38 ± 1.96	7.75 ± 2.01	4.00 ± 0.98	3.75 ± 1.11
NAL (n=6)	14.17 ± 1.89	5.33 ± 1.50	5.33 ± 0.95	3.83 ± 1.05
TME (n=6)	18.33 ± 1.28	13.00 ± 1.03	8.50 ± 0.77	6.29 ± 1.25

SCOP: Scopolamine (3 mg/kg, i.p.); FMZ: Flumazenil (2 mg/kg, i.p.); NAL: Naloxone (2.5 mg/kg, i.p.); TME:Thiamine (1 mg/kg, i.p.).

*p<0.05 vs vehicle; **p<0.05 vs corresponding extract dose level.

Table 4: Effects of aqueous extract of *Anaphe venata* on ataxia behaviour in mice and the involvement of cholinergic, GABAergic, opioidergic systems.

	Vehicle	Aqueous Extract of <i>Anaphe venata</i> (mg/kg, i.p.)		
		100	200	400
Vehicle (n=12)	0.00 ± 0.00	15.17 ± 3.89	15.83 ± 4.71*	9.17 ± 2.77*
SCOP (n=12)	0.00 ± 0.00	0.00 ± 0.00**	0.00 ± 0.00**	0.00 ± 0.00**
FMZ (n=6-8)	0.00 ± 0.00	10.88 ± 5.04	11.00 ± 2.99	7.88 ± 3.73
NAL (n=6)	0.00 ± 0.00	2.33 ± 1.20	3.17 ± 2.41	8.17 ± 2.74
TME (n=6)	0.00 ± 0.00	27.67 ± 6.96	42.83 ± 7.24**	52.33 ± 7.26**

SCOP: Scopolamine (3 mg/kg, i.p.); FMZ: Flumazenil (2 mg/kg, i.p.); NAL: Naloxone (2.5 mg/kg, i.p.); TME:Thiamine (1 mg/kg, i.p.).

*p<0.05 vs vehicle; **p<0.05 vs corresponding extract dose level.

naloxone (μ -opioid receptors antagonist) and thiamine did not have any effect on the inhibitory effect of extract on rearing. This suggests that GABAergic and opioid neural systems are not involved in the inhibitory effect of the extract on rearing.

Anaphe extract induced stretching behaviour in rats when compared to saline control and this was significantly reversed by scopolamine therefore suggesting the involvement of muscarinic-cholinergic system in the observed extract-induced stretching behaviour. This suggestion is supported by the fact that cholinergic neurotransmission was reported to play a significant role in 5-HT₆ antagonist-induced stretching behaviour in rats that was abolished by pretreatment with muscarinic antagonists (Bentley *et al.*, 1999). Further more, it was observed that stretching induced by the aqueous extract was potentiated by flumazenil, naloxone and thiamine. It could therefore be suggested that the GABAergic, opioid and cholinergic systems are involved in the stretching phenomenon. The role of thiamine may not be unconnected with its involvement in the presynaptic release of acetylcholine centrally and therefore a manifestation of its central cholinomimetic effect and anticholinestrase action (Meador *et al.*, 1993).

Anaphe extracts significantly induced ataxia behaviour in rats when compared with saline control. This effect was significantly blocked by scopolamine, naloxone, and thiamine, which suggested the involvement of cholinergic, GABAergic and opioidergic neural systems in the ataxia phenomenon. Cholinergic mechanism has previously been implicated in motor inco-ordination (Fuhmann *et al.*, 1985) and that central opiate receptor mechanism has been reported to play a role in ataxia (Castellani *et al.*, 1982) where naloxone was observed to significantly decrease phencyclidine-induced stereotypy and ataxia behaviours. Thus, the complete blockade of *Anaphe venata* ataxia-induced behaviour clearly showed that muscarinic-cholinergic receptors are highly

involved in this phenomenon and therefore muscarinic-cholinergic antagonist drugs may be useful in the management of *Anaphe venata*-induced ataxia behaviour. Furthermore, it also showed the involvement of central GABAergic and opiate receptors mechanisms in the mediation of aqueous extract of *Anaphe venata*-induced ataxia behaviours in the rat. Thiamine was observed to potentiate the ataxia-induced by the administration of aqueous extracts of *Anaphe venata* and the results are therefore consistent with a cholinomimetic effect of thiamine as earlier reported in animal (*in vitro*) studies where thiamine was found to be involved in the presynaptic release of acetylcholine and also exhibited anticholinesterase activity (Meador et al., 1993).

Amphetamine is a central stimulant drug whose behavioural actions in laboratory animals are thought to be due to facilitation of dopaminergic transmission (Parker & Cubeddu, 1986; Hurd & Herkenham, 1992; Wang *et al.*, 1995). The aqueous extracts significantly suppressed amphetamine-induced stereotypy and locomotion in extract pretreated rats compared to control suggesting that this extract is also probably acting via dopaminergic system.

In conclusion, these results showed that these *anaphe*-induced behavioural effects are mediated via cholinergic, GABAergic, opioidergic and dopaminergic receptor systems with strong muscarinic-cholinergic receptors involvement in ataxia-induced behaviour. We therefore suggest that muscarinic-cholinergic like drugs may be of benefit in the management of patients that present with clinical condition of seasonal ataxia.

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