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NEUROPHARMACOLOGICAL STUDIES OF DREVOGENINS ISOLATED FROM *WATTAKAKA VOLUBILIS* (LINN.F.) STAPF. IN MICE

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ABSTRACT

The *Wattakaka volubilis* ethanolic extract (WVEE), *Wattakaka volubilis* sapogenin mixture (WVSM) and drevogenins (DVG) isolated from *Wattakaka volubilis* (Linn.f) Stapf. were evaluated for neuropharmacological activities using spontaneous motor activity, rotarod performance, motor performance evolution and muscle relaxant test models in mice. Sapogenins seemed to be responsible for neuropharmacological activities in the studied models. When compared to the saline treated control WVEE at a dose level of 200 mg/kg, WVSM at a dose level of 50 mg/kg and DVG I and II at a dose level of 20 mg/kg body weight significantly increased (p<0.001***) the gross behavioral effect such as gripping strength, muscle tone and muscle coordination.

KEY WORDS: ***Wattakaka volubilis,*** **Drevogenins, motor performance evaluation, muscle relaxant test, neurosteroids**

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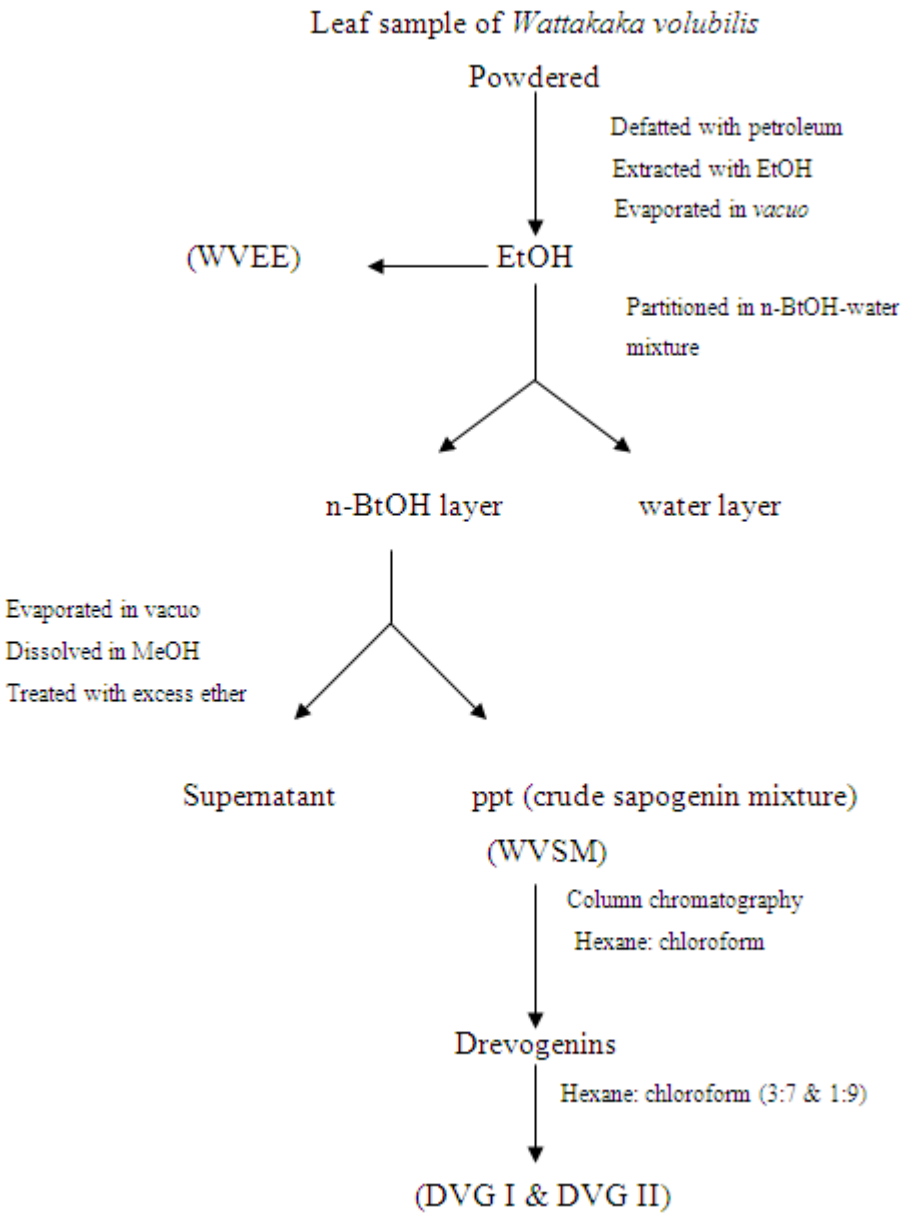
INTRODUCTION

Wattakaka volubilis Linn.f. (Satf.f.) (syn. *Dregea volubilis)* is commonly found in India and South East Asia. It is a climber with green flowers in drooping umbel with smooth bark and ash coloured leaves rounded at the base. In Ayurveda, *Wattakaka volubilis* is extensively used to treat inflammation, piles, leucoderma, asthma, tumors, urinary dis-charge etc. (Kirtikar and Basu, 1935). Drevogenin D and kaempferol have been isolated from its leaves whereas dregeo-sides, hyperoside, drevogenin A and P as also drebbysogenin were isolated from its seeds, stem and roots respectively (Anonymous, 1976; Yoshimura et al., 1983, 1985). Furthermore, Sahu et al. (2002) isolated three novel polyoxypregnane glycosides volubil-ioside A, B, C along with drevogenin D and P from the flowers. Moreover, this plant is used as a remedy for sore throat, abdominal tumours. It is used as an appetizer, carminative and aphrodisiac (Agarwal V S). The alcoholic extract of the plant has effect on central nervous system. It has also shown anticancer activity against sarcoma 180 in the mice (Tteng, N.S., 1883). The young roots are cut and exuding juice is inserted into the nose to cause sneezing. The purpose of the present work was to investigate the neuropharmacological potency of ethanolic, sapogenin mixture and drevogenins of *Wattakaka volubilis.*

MATERIALS AND METHODS

Wattakaka volubilis Linn.f. (Satf.f.) leaves were collected from Khanapur forest, Bidar, Karnataka, India during August- September 2009. Authentication was done by Y.N. Seetharam, faculty of Botany, Gulbarga University, Gulbarga, where a voucher specimen has been deposited in the herbarium (HGUG No. 83). About 500 g of dried leaves of *Wattakaka volubilis* were reduced to fine powder which was subjected to various treatments as shown in Fig.1 (Yosioko I et al., 1974). 5 gm of the *Wattakaka volubilis* sapogenin mixture (WVSM) was loaded on to the column and was eluted with the different proportions of hexane and chloroform. The fraction 3:7 and 1:9 obtained was subjected to determination of melting point and spectral analysis mainly 1D and 2 D NMR studies.



Animals

Swiss albino mice 18 – 30 g (n=36) of either sex (male=24 & female=12) were procured from Mahaveer Enterprises, Hyderabad, India and were acclimatized at central animal house, Luqman College of Pharmacy, Gulbarga. They were housed in appropriately designed cages (28x20x14). The animals were maintained under standard husbandry conditions, temperature 22±2ºC, relative humidity 40 % for 12 h day and night illumination cycle. The animals were fed *ad libitum* with standard pellets (VRK Nutritional Solutions Sangli, Maharashtra, India) and had free access to water. CPCSEA (Committee for the purpose of control and supervision on experiments on animals) guidelines were followed throughout the study and the protocol was approved by the IAEC (Institutional animal ethical committee) No. 346 Luqman College of Pharmacy, Gulbarga.

Experimental study

Animals (male mice) were divided into six groups and they were housed 6 per cage. The first group served as the vehicle treated control

(saline), the group II served as standard (Diazepam (20 mg/kg i.p), where as groups III, IV, V, and VI were administered with WVEE (200 mg/kg), WVSM (50 mg/kg), DVG-I (20 mg/kg) and DVG-II (20 mg/kg) respectively. Acute toxicity studies were carried out as per the OECD guidelines.

Neuropharmacological studies

Motor performance by spontaneous motor test

This study was performed using acto-photometer, which had photoelectric cells connected in circuits with a counter, when the beam of light falling on the photocell was cut off by the animal movement in the chamber a count was recorded. Mice belonging to each group individually kept for 5 min in the instrument and readings were taken at 0, 1, 2, 4 and 8 hours of administration of the doses (Gamaniel et al., 1998).

Motor performance by rota-rod test

A rota-rod trade mill device was used to assess the locomotory activity. Animals were placed on a horizontal rotating rod with diameter of 5 cm set at 16 rpm. 60 min after treatment each mouse was placed on the rotating rod for 300 sec at different intervals of 2, 4, 8 hours. Falling of animal within 300 sec indicated lack of motor coordination (Fujimori and Cobb, 1965).

Motor performance by horizontal wire test

Gripping strength effect was evaluated using the horizontal-wire test which consisted of a stretched copper wire placed 20 cm above the ground (Boissier *et al.*, 1961). The animal was suspended in the wire by its fore paws and the time taken for the animal to reach the wire with the hind paws or tails were counted. Animals which failed to do it within 5 sec were considered to have failed the test and this was considered to be synonymous with muscle relaxation (Vogel and Vogel, 1997).

Muscle relaxant test

Motor coordination was assessed using the chimney test which consisted of a simple glass tube in which the experimental mouse entered and when it reached the other end, the tube was placed in a vertical position. The normal reaction of the animal was to climb the tube backward. Animal performance was evaluated by the time taken to reach the upper edge of the glass tube considering motor impairment the inability of the mouse to climb backwards up the tube within 30 sec (Boissier *et al.*, 1961).

Statistical analysis

Statistical analysis of results obtained was done with GraphPad Insat® software, using ordinary ANOVA followed by post-hoc Tukey's test. The statistical values are expressed as P values where $p<0.001$*** highly significant, $p<0.01$** moderately significant, $p<0.1$* significance.

RESULTS

Drevogenin extraction

The phytochemical analysis of the colourless crystals formed in the fraction 3:7 and 1:9 revealed the presence of sapogenins by answering the Libermann-Burchard test for triterpenoids. The purity of isolated compounds was checked through HPLC and was found to be free from impurities. The molecular formula of the Drevogenins was determined as $C_{16}H_{30}O_6$ by MS-MS (positive mode found *m/z* 471.2713, calcd for [M + Na] 471.2723) and 13C NMR spectral data. The IR spectrum showed absorption bands at 3432 and 1686 cm^2 for hydroxyl and carbonyl groups respectively. The 13 C NMR spectrum revealed the presence of five methyls, six methylenes, nine methines, and six quarternary carbons including signals appropriate for two tri-substituted double bonds and an ester function. From these results, the

isolated compound was confirmed as Drevogenins (Fig 5).

Acute toxicity studies

WVEE (250-1000 mg/kg. p.o), WVSM (50-300 mg/kg. p.o), DVG-I and DVG-II (5-30 mg/kg. p.o) were well tolerated by the animals, as no death was observed over a period of 7 days post treatment. However, at higher dose all the treated groups produced lethal effect of 50 %.

Neuropharmacological studies

Fig 1 represents spontaneous motor activity assessed by acto-photometer. There was a significant increase in the movement of the animals as recorded by an increase in the digital reading. When compared to vehicle treated control the WVEE had showed significant increased motor activity at $p<0.01$** while the WVSM, DVG-I and DVG-II showed the increased motor activity at $p<0.001$***. The test for motor coordination revealed the gripping strength of the mice placed on the rota-rod where the time spent on the rotating rod was measured (Fig 2). When compared to the vehicle treated control WVEE, WVSM showed an increased gripping strength with a significant P value of $p<0.01$** while the DVG-I and DVG-II showed significant P values at $p<0.001$***. In the horizontal wire test where the animals were suspended on the horizontal wire with their fore paw and the time taken to hold the hind paw or tail was counted (Fig 3). When compared to vehicle treated control the WVEE and DVG-I had showed increased motor strength with a significant value of $p<0.01$** where as the WVSM and DVG-II showed the significant P value $p<0.001$*** The muscle relaxant test revealed the motor impairment of the mouse to climb backward the tube within 30 sec (Fig 4). When compared to the vehicle treated control all the treated groups were able to climb the chimney within the threshold time at the P value of $p<0.001$***

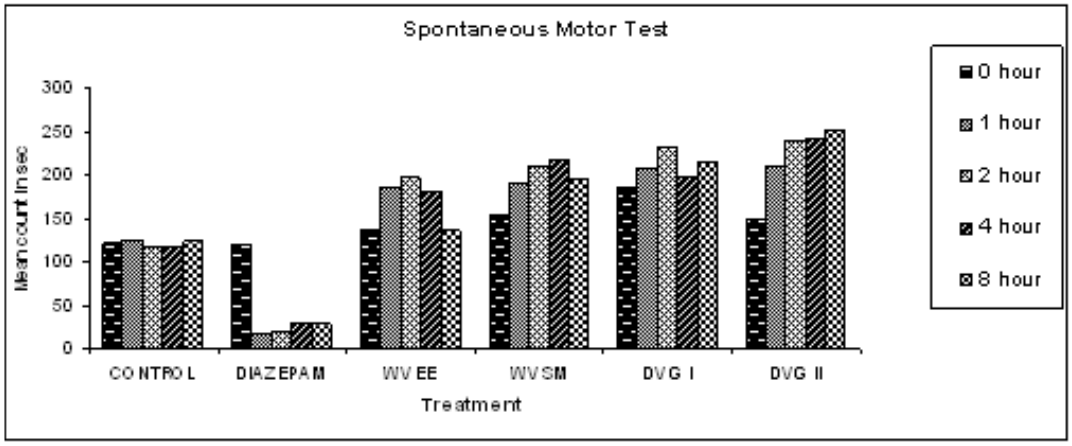


Figure 1

Analysis of behaviour of mice in the spontaneous motor activity assessed by actaphotometer as a result of excitability of CNS. Results expressed as Mean (±SEM) number of counts in movement of animals in 5 min. p<0.001, p<0.01**, p<0.5* are significantly different from control (ANOVA; Tukey's test).***

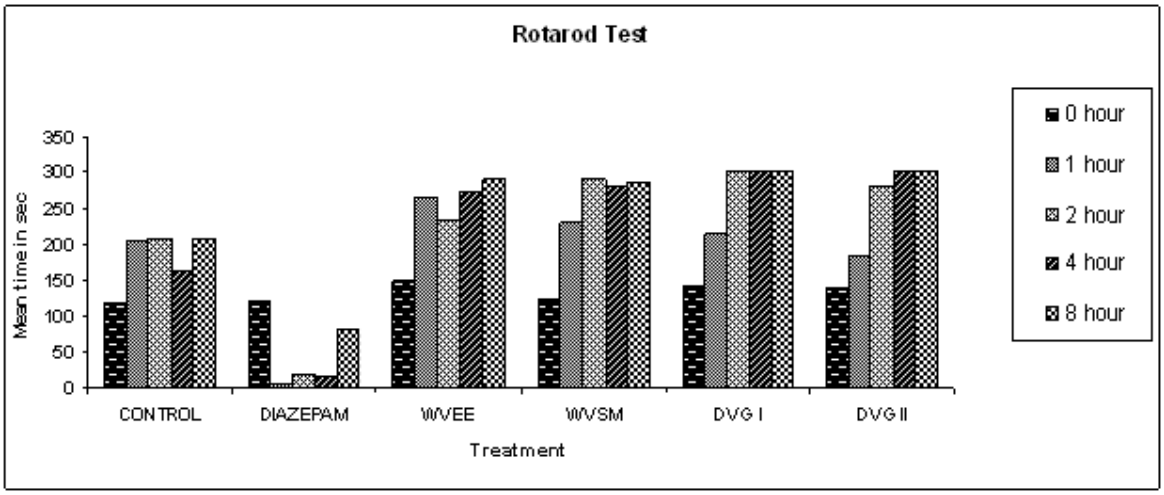


Figure 2

Analysis of behaviour of mice in the motor coordination assessed by Rota rod performance as a result of peripheral and CNS mediated effects. Results expressed as Mean (±SEM) gripping strength till 5 min. p<0.001, p<0.01**, p<0.5* are significantly different from control (ANOVA; Tukey's test).***

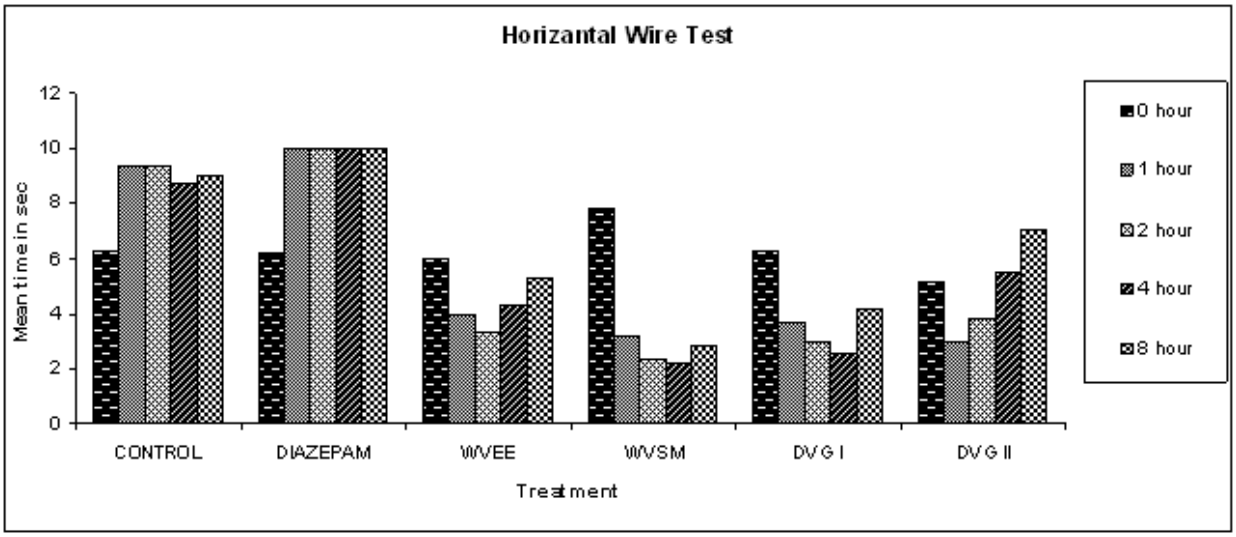


Figure 3

Analysis of behaviour of mice in the horizontal wire test as a result of muscle tone. Results expressed as Mean (±SEM) decrease in the time to hold the wire with hind paw or tail in 5 sec. p<0.001, p<0.01**, p<0.5* are significantly different from control (ANOVA; Tukey's test).***

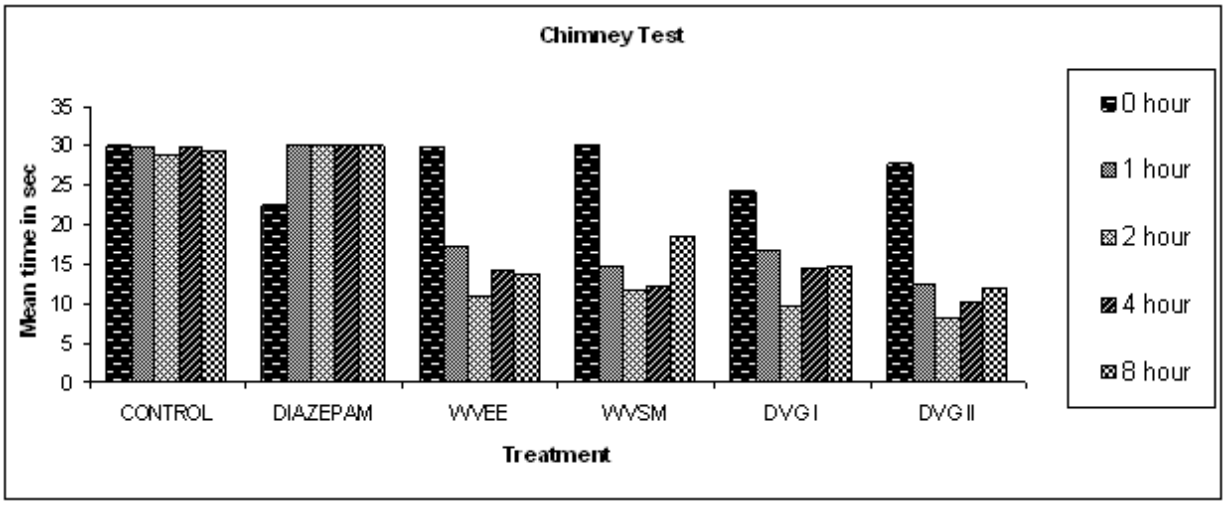
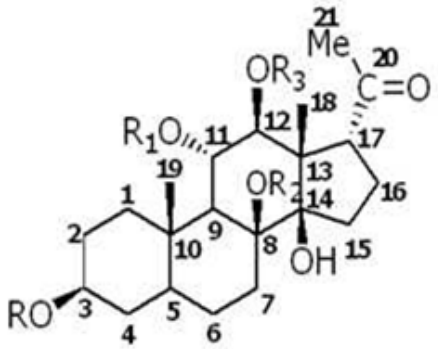


Figure 4

Analysis of behaviour of mice in chimney test as a result of muscle tone. Results expressed as Mean (±SEM) increase in the climbing of animals in 30 sec. p<0.001, p<0.01**, p<0.5* are significantly different from control (ANOVA; Tukey's test).***

Fig 5

Chemical structure of Drevogenins



1. ***DVG I, R= allomethyllose-thevetose; R¹&R²= Tigloyl; R³= Benzoyl.***
2. ***DVG II, R= H; R¹= Tigloyl; R²& R³= OH***

DISCUSSION

The spontaneous motor activity is a model, which has been used in the laboratory animals to evaluate the gross behavioral effects of drugs (Hsieh et al., 1991). The model measures the excitability of the central nervous system (Mansur et al., 1971) which correlates well with drug effects in humans. Our study shows significant (p<0.001***) increase in the spontaneous motor activity with the treated groups WVSM, DVG-I and DVG-II which may be due to an induction in the excitability of the central nervous system which could be suggestive of anti-anxiolytic activity, where as the saline treated showed normal motor activity. The test for motor coordination rota rod performance was adopted to evaluate the

effect of the *Wattakaka volubilis* and its constituents on the physical performance, endurance and possible neuromuscular inhibitions. The isolated compounds DVG-I and DVG-II produced significant effect at $p<0.001$** on motor coordination suggesting the peripheral neuromuscular activation through centrally mediated effect. The horizontal wire test and chimney test were performed to evaluate the muscle tone that accompanies various neurological disorders and injuries of the brain and spinal cord as well as states of anxiety (Bohlhalter et al., 1996). In our study there was an increase in muscle tone as the animals were able to grasp the wire with their hind limb and tail within the prescribed time, whereas the diazepam treated animals have shown sedative effect with the horizontal wire test. The saline treated animals showed normal muscle tone. According to Crestani et al., (2001) the myorelaxant effect of diazepam is largely mediated via α2 GABAA receptors those expressed on motor neurons and in the superficial layer of the dorsal horn although supraspinal α2 GABA-A receptors may also be involved. Motor coordination was assessed using the chimney test considering motor impairment by inability of the mouse to climb backwards the tube within 30 sec (Coleta et al., 2006). In our study there was a significant increase in the motor coordination performed by chimney test where the time taken to climb the chimney was significantly reduced, while the diazepam treated animals failed in answering due to sedative effect mediated through benzodiazepine receptors.

According to Clark et al (2004) pregnenalone and allopregnenalone which are chemically 3α-hydroxy-5α-pregnan-20-one mainly act as negative modulators of GABA-A receptor activity and positive modulators of NMDA receptors. They also play an important role in the control of neural development and the improvement of neuron plasticity. Allopregnenalone also modulates the GABA-A activity and its physiological role is important in neurogenesis, survival and migration of neurons. In addition alloprenenalone are positive allostearic modulators of the GABAA receptor that augment channel burst duration by increasing the opening frequency without concomitant changes in the open duration time (Henderson and Jorge, 2004). The neurosteroids pregnenolone and allopregnenolone can both potentiate and antagonize GABAA receptors mediated current depending on the subunit composition of the receptor and the functional group of the neurosteroid (Jorge –Rivera et al., 2000). Polyhydroxy pregnane glycosides of *Wattakaka volubilis* are chemically similar to the neurosteroids pregnenolone and allopregnenolone and they may act as the negative modulators of GABA-A receptor and positive modulators of NMDA receptors resulting in the CNS stimulant activity.

CONCLUSION

In conclusion, this investigation provides evidence that may support the ethno medical applications of the drevogenins isolated from the leaves of *Wattakaka volubilis* in the treatment of neurological disorders. The study also reveales that drevogenins are chemically similar to the brain neurosteroids possess motor performance strength and muscle relaxant activity. However, further studies are necessary to clearly define mechanism responsible.

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