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Anticonvulsant and depressant-like activity of ursolic acid stearoyl glucoside isolated from *Lantana camara* L. (verbanaceae)

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ABSTRACT

Objective: Ursolic acid stearoyl glucoside (UASG), a new terpenoid -isolated from Lantana camara L. was evaluated for its anticonvulsant and depressant activity of was in Wistar albino rats and Swiss mice. **Methods:** Column chromatography was used to isolate UASG. The intraperitonial administration of UASG (25 and 50 mg/kg) produced a significant depressant effect on CNS. Anticonvulsant potential was experimentally proved and demonstrated through Maximal electroshock (MES) induced seizure, Isoniazid (INH) induced seizure and Assessment of locomotor activity test. **Results:** Administration of drug reduced spontaneous motor activity ($P<0.05$), the number and lethality ($P<0.01$ and $P<0.001$) of Isoniazid (INH)-induced seizures and inhibition of hind limb extension in ($P<0.05$ and $P<0.001$) in Maximal Electroshock (MES)-induced seizures. **Conclusions:** Research finding suggests that ursolic acid stearoyl glucoside possess anticonvulsant and depressant like effect.

1. Introduction

Lantana camara L. is a well known tropical plant [1]. Ursolic acid is one of the best known bioactive pentacyclic triterpenoids. It is widely distributed in nature in many plant species and used as medicinal plants in traditional medicine and also exist in food products [2], creating great interest to scientists due to its several biological activities. These include anti-inflammatory [3], antiobesity [4], cytotoxic [5], antiproliferative, antimicrobial [6], and antidiabetic effect [7]. In view of its easy availability in many plants and long term treatment of CNS disorders in modern medicine, we have carried out a detailed evaluation of its anticonvulsant and CNS depressant activity.

Ursolic acid obtained from Salvia officinalis reported for anti-inflammatory, antioxidative, antiprotozoal, antimutagenic and anticancer properties [8]. Awad et al in 2009 reported in-vitro assays of ursolic acid inhibited GABA-T by 20% at 100ug/ml of dose [9]. The traditional uses of Lantana camara L. mainly refer for the treatment of asthma, ulcers, measles, chickenpox, eczema, tumors, cancers, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria, ataxy of abdominal viscera [10], memory weakness, enhance intellect and cognition [11]. The plant has been reported for its anticancer [12-13], antiulcer [14], antioxidant [13], anti-diabetic [15], antifungal, antibacterial [16], anti-feedant, larval mortality/repellency [17], anti-motility [18], analgesic and anti-inflammatory [19] activities.

The present study was designed to evaluate the anticonvulsant potential of UASG isolated from Lantana camara L. against maximal electroshock (MES) induced and isoniazid (INH) induced seizures in experimental animals. Moreover sedative effects of the UASG were evaluated.

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2. Materials and Methods

2.1 Plant material

Lantana camara L. leaves were collected from Herbal Garden of Siddhartha Institute of Pharmacy, Dehradun and identified by Dr. S. B. Singh, Scientist, NISCAIR, New Delhi. A voucher specimen (NISCAIR/RHMD/consult/-20-09-10/1322/125) was deposited in the herbarium of NISCAIR, India.

2.2 Extraction and isolation of UASG

Dried powder of Lantana camara leaves (4 kg) was extracted with methanol (12 L) at 50 ℃ for 1 day. Extract was concentrated to dryness under reduced pressure to obtain slurry (605 g). The slurry was dissolved in minimum amount of methanol and was adsorbed on silica gel (60-120 mesh). The slurry was subjected to a silica gel column using $CHCl_3$/ MeOH gradient system (49:1; 2.0 L for gradient system); leads to elution of colorless crystals of USAG (yield 11.2 g, 0.28 %). Structure of compound was identified by comparison of their spectroscopic data from the reported literature [7]. The structure of USAG is depicted in Fig. 1.

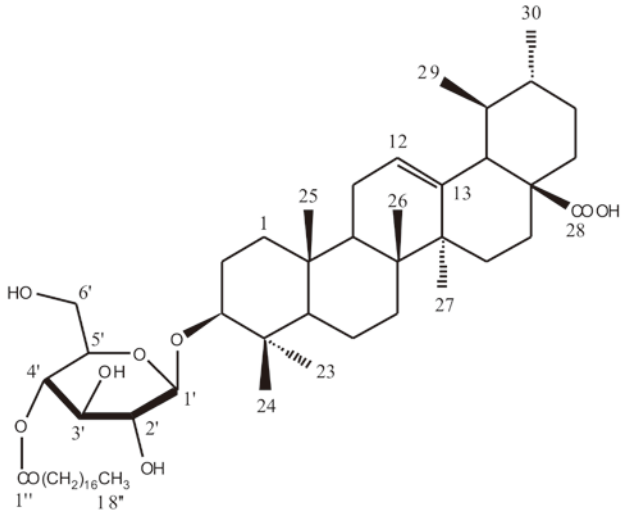

Fig 1. Structure of ursolic acid stearoyl glucoside

2.3 Animals

Wistar albino rats (150-200 g) and Swiss mice (20-25 g) were obtained from Central Animal Facility, Hamdard University and kept at 25 ± 1 ℃, 55 ± 5 % humidity along with 12 hr light/ dark cycle. The animals were given standard pellet diet (Lipton rat feed, Ltd., Pune) and water ad libitum throughout the experimental period. The experiment was approved by the 'Institutional Animal Ethics Committee'. UASG and the standard drug were administered intraperitonially .

2.4 Chemicals

Isoniazid, Diazepm and Phenytoin were purchased from Himgiri Traders, Dehradun. All other chemicals used were of analytical grade. Isoniazid was dissolved in normal saline and UASG was suspended in saline and used.

2.5 Maximal electroshock (MES) induced seizure

Wistar albino rats (150-200 g) were divided into four groups with six animals in each group. Group I was served as solvent control and received 0.9% (w/v) of saline (1 ml/100g). Group II was treated as positive control, received phenytoin (20 mg/kg). Group III and IV received UASG, 25 and 50 mg/kg suspended in 0.9% (w/v) of saline. All the treatments were administered intraperitonially 30 min prior to the electroshock. The electroshock was induced in animals by passing a current of 150 mA for 0.2 s duration through auricular electrodes. After electric stimuli, latency and incidence of tonic hind limb extension (THLE) and mortality was observed for duration of 15 min. Protection was defined by complete absence of tonic hind limb extension.

2.6 Isoniazid (INH) induced seizure

Wistar albino rats (150-200 g) were divided into four groups with six animals in each group. Group I was served as solvent control and received 0.9% (w/v) of saline (1 ml/100g). Group II was treated as positive control, received Diazepam (5 mg/kg). Group III and IV received UASG, 25 and 50 mg/kg suspended in 0.9% (w/v) of saline. All the treatments were administered intraperitonially 30 min prior to the administration of INH (300 mg/kg). Animals that did not convulse within 30 min were considered as protected. The number of rat protected in each group was expressed in terms of percentage. In the INH treated group, the animals were monitored for 60 min, and the percent protection was determined. In unprotected animals, the latency to first convulsion and the durations of convulsions were recorded. The animals were observed for 24 h after administration of INH for their mortality rate.

2.7 Assessment of locomotor activity

Swiss mice (20-25 g) were acclimatized with environment and placed individually in a digital actophotometer (INCO, Ambala, India). The locomotor activity of the animals was evaluated after 30 min of drug administration. Each animal was observed over a period of 5 min in a square (30 cm) closed arena equipped with infrared light-sensitive photocells and the values expressed as counts per 5 min, subsequently they were divided into four groups and all the groups were treated as per INH induced seizure model. The locomotor activity is an index of wakefulness (alertness) and is use to assess the sedative effect of the drugs.

2.8 Stastistical analysis

The data were expressed as mean ± S.E.M. Statistical comparisons were performed by one-way ANOVA followed by Tukey's post-test using Graph Pad Prism version 5.0, USA. $P<0.05$ was considered significant.

3.Results

3.1 Behavioural and toxic effects

UASG was administered to the test groups in graded doses ranging up to 100 mg/kg body weight/day and the rats were observed 10 days for any signs of mortality and behavioural disabilities. Then dose was increased upto 500 mg/kg and again observed for signs of mortality and behavioural disabilities for another 10 days. Its LD50 value was found to be higher than 500 mg/kg body-weight in rats. 1/10th and 1/20th of 500 mg/kg of UASG was used for the further experimentation.

The duration of THLE for the control group was 10.31±0.60 s after an electroshock in MES model. Administration of UASG (25 and 50 mg/kg, i.p.) showed significant (P<0.05 and P<0.001) reduction in the duration of THLE to 6.59±1.35 s and 0.40±0.32 s, respectively when compared to the control group (Table 1). At 25 and 50 mg/kg dose of UASG, it exhibited 16.66 and 83.33% protection against mortality. However, the standard phenytoin 20 mg/kg showed to absence of convulsions and 100% protection against mortality.

The latency and duration of seizure induced by INH in the control animals was found to be 160.2±23 s and 46.50± 0.96 s respectively (Table 2). UASG at 25 and 50 mg/kg doses significantly (P<0.001) increase latency of seizure and decrease duration of seizure. At 25 mg/kg dose it exhibited 33.3 % mortality in animals, while at 50 mg/kg and standard diazepam 5 mg/kg protects all the animals and the mortality rate was 0.00 %.

UASG at both doses and standard diazepam 5 mg/kg, significantly (p<0.001) reduced the locomotor activity in mice when compared to the control animals (Table 3). At 50 mg/kg dose it showed better reduction in locomotor activity (60.62%) when compared to standard diazepam (54.85%).

4. Discussion

The MES is a standard procedure that evaluates the testing materials ability to protect against THLE in MES. Toman et al. (1914) reported that the seizure pattern in MES for all laboratory animals and man are similar except for time scale. Protection against THLE in the MES predicts anticonvulsant activity of anticonvulsant drugs that prevent the spread of the epileptic seizure from an epileptic focus during seizure activity. Protection against THLE also indicates the ability of the testing material to inhibit or prevent seizure discharge within the brain stem substrate [20]. Since, the UASG showed anticonvulsant activity in the MES, it may act through any of the above mentioned mechanisms.

The convulsant action of INH involves disruption of GABAergic neurotransmission in the CNS. It is well documented that INH inhibits GAD, an enzyme that catalyzes the synthesis of GABA from glutamic acid. Several anticonvulsant drugs in current clinical use facilitate GABA neurotransmission by different mechanism: barbiturates,

Table 1
Effect of ursolic acid stearoyl glucoside on MES-induced seizure in rats.

Treatment (dose,mg/kg, *i.p.*)	No. convulsed/no.used	Animals not convulsed(*i.e.* % animals protected)	Duration of tonicconvulsions (sec)Mean ±SEM	Mortality(% Death)
MES Control	6/6	0	10.31±0.60	3/6 (50.0)
Phenytoin (20)	0/6	100	Absence of extension	0/6 (0.00)
UASG (25)	5/6	16.66	6.59±1.35a	0/6 (0.00)
UASG (50)	1/6	83.33	0.40±0.32c	0/6 (0.00)

All values are mean ± SEM; n=6.
a P<0.05, c P<0.001, when compared to control.

Table 2
Effect of Ursolic acid stearoyl glucoside on INH-induced seizure in rats.

Treatment (dose,mg/kg, i.p.)	No. convulsed/no.used	Animals not convulsed (i.e. % animals protected)	Seizure latency (s)	Seizure duration (s)	Mortality(% Death)
INH Control	6/6	0	160.2±23	46.50±0.96	6/6 (100)
Diazepam (5)	3/6	50	344.7±30c	21.17±0.48c	0/6 (0.00)
UASG (25)	6/6	0	180.2±35b	39.22±0.31c	2/6 (33.3)
UASG (50)	4/6	66.67	325.4±37c	24.18±0.48c	0/6 (0.00)

All values are mean ± SEM; n=6.; b P<0.01, c P<0.001, when compared to control.

Table 3
Effect of Ursolic acid stearoyl glucoside on locomotor activity in mice.

Treatment (dose, mg/kg, i.p.)	Locomotor activity (Score) in 5 min (Mean±SEM)		Reduction in activity(%)
	Before treatment	After treatment	
Normal Control	272.5±7.19	233.8±5.97	14.34
Diazepam (5)	237.8±17.70	107.7±4.10a	54.85
UASG (25)	244.3±19.19	184.0±3.39a	24.59
UASG (50)	259.0±12.00	102.8±4.43a	60.62

All values are mean ± SEM; n=6; a P<0.001, when compared to control.

benzodiazepines and other anticonvulsants modulate the action of GABA by enhancing chloride currents in channels linked to different receptor sites [21]. The effect of most of anticonvulsant agents is to enhance the response to GABA, by facilitating the opening of GABA-activated chloride channels. GABAA receptors were involved in epilepsy and their direct activation would have an anticonvulsant effect. Therefore UASG might possibly be producing anticonvulsant action by increasing the level of GABA, an inhibitory transmitter in the central nervous system. This is in accord with the pharmacological effects of benzodiazepine and highlights the relevance of the putative anticonvulsant effects of UASG.

The locomotor activity test correlates the sedative effect of UASG. Locomotor activity is considered as an index of alertness its decrease is indicative of sedative activity. The anticonvulsant effects of drugs such as benzodiazepines are accompanied by decreased locomotor activity and sedation. The results indicates that UASG inhibited locomotor activity to more significant level than diazepam, and thus has a better profile for an anticonvulsant effect [22].

From above results, the present study reveals that the ursolic acid stearoyl glucoside obtained from *Lantana camara* L. exhibited anticonvulsant and sedative properties. These neuropharmacological properties are possibly mediated via facilitation of GABA transmission.

Further studies are required on ursolic acid to elucidate the possible mechanism involved and its use in human beings.

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Conflict of interest statement

We declare that we have no conflict of interest.

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