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LETTER TO THE EDITOR

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Antiepileptic Potential of Ursolic Acid Stearoyl Glucoside by GABA Receptor Stimulation

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The antiepileptic potential of ursolic acids stearoyl glucoside (UASG) isolated from *Lantana camara* L. was evaluated against maximal electroshock (MES) induced and isoniazid (INH) induced seizures in experimental animal models. Ursolic acid is already known for CNS depressing, anticonvulsant and analgesic activity [1]. Dried powder of *Lantana camara* leaves (4 kg) was extracted with methanol (12 L) at 50°C for 24 h. The slurry obtained was dissolved in methanol, and further, it was adsorbed on silica gel (60–120 mesh). The slurry was subjected to a silica gel column using CHCl3/MeOH gradient system (49:1; 2.0 L for gradient system); it yielded colorless crystals of USAG (yield 11.2 g, 0.28%) [2]. Wistar albino rats (150–200 g) were divided into four groups (n = 6). Group I (solvent control), received 0.9% (w/v) of saline (1 mL/100 g), Group II (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. Antiepileptic potential of UASG was established through INH and MES protocol–induced seizure.

Isoniazid-induced seizure model, all animals were treated intraperitonially 30 min prior to administration of INH (300 mg/kg). Animals that did not convulse within 30 min were considered as protected and their results were expressed in terms of percentage. Group III and IV animals were monitored for 60 min for determination of percentage protection. In unprotected animals, the latency to first convulsion and the durations of convulsions were recorded [3]. Forty-five minute after vehicle or compound **1** and 30 min after diazepam, rats were sacrificed. The animals of group III were sacrificed as soon as onset of convulsions occurs or 65 second after INH treatment. Brain tissue was isolated and transferred

Table 1 Effect of Ursolic acid stearoyl glucoside on isoniazid (INH)-induced seizure in rats

Treatment (dose, mg/kg, i.p.)	No. convulsed/ no. used	Animals not convulsed (% animals protected)	Seizure latency (s)	Seizure duration (s)	Mortality (%)	GABA level (ng/g of brain tissue)
INH control	6/6	00.0	160 ± 23	46.5 ± 0.96	6/6 (100)	29.9 ± 0.87
Diazepam (5)	3/6	50.0	345 ± 30**	21.2 ± 0.48**	0/6 (0.00)	46.0 ± 1.22**
UASG (25)	6/6	00.0	180 ± 35*	39.2 ± 0.31**	2/6 (33.3)	36.8 ± 1.09*
UASG (50)	4/6	66.7	325 ± 37**	24.2 ± 0.48**	0/6 (0.00)	46.2 ± 1.45**

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

Table 2 Effect of ursolic acid stearoyl glucoside on maximal electroshock (MES)-induced seizure in rats

Treatment (dose, mg/kg, i.p.)	No. convulsed/ no. used	Animals not convulsed (i.e.,% animals protected)	Duration of tonic convulsions (second) Mean ± SEM	Mortality (%)
MES control	6/6	00.0	10.30 ± 0.60	3/6 (50.0)
Phenytoin (20)	0/6	100	Absence of extension	0/6 (0.00)
UASG (25)	5/6	16.7	6.59 ± 1.35*	0/6 (0.00)
UASG (50)	1/6	83.3	0.40 ± 0.32**	0/6 (0.00)

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

to homogenization tube, containing 10 mL of 0.01 M hydrochloric acid. Homogenate was transferred in a bottle with 16 mL of ice-cold absolute alcohol. The samples were subjected to centrifugation at 10,573 ***g*** for 10 min to obtain the precipitate. Precipitate was washed thrice with 10 mL of 90% alcohol. Washed liquids were combined with supernatant. The combination was transferred to petri plate for evaporation and dried at 70°C. 2 mL water and 4 mL of chloroform added to the dry mass and centrifuged at 182 ***g***. Upper phase containing GABA (4.0 mL) was separated and 20 μL of it was spotted on Whatman paper (No. 41). n-butanol (50 mL) acetic acid (12 mL) and water (60 mL) were selected as mobile phase. Ascending technique was adopted to develop the paper chromatogram. 0.5% ninhydrin solution in 95% ethanol was sprayed and it was dried for 1 h at 90°C. Blue color spot was developed, cut and heated with 2 mL ninhydrin solution on water bath for 5 min. Water (10.0 mL) was added to solution and kept for 1 h. Supernatant (4.0 mL) was decanted and absorbance was measured at 570 nm [4].

Ursolic acids stearoyl glucoside at 25 and 50 mg/kg doses significantly ($P < 0.001$) increase latency and decrease duration of seizure in INH-induced seizure animal model. At 50 mg/kg UASG and standard diazepam 5 mg/kg protects all the animals, and the mortality rate was 0.00%. Compound **1** exerted a positive effect on GABA level, that is, the level of GABA was found to be significantly increased up to a dose of 50 mg/kg (Table 1). The convulsant action of INH involves disruption of GABAergic neurotransmission in the CNS by inhibiting glutamic acid decarboxylase, an enzyme that catalyzes the synthesis of GABA from glutamic acid. Several anticonvulsant drugs in current clinical used to facilitate GABA neurotransmission by different mechanisms [5]. Hence, compound **1** reduces epileptic seizures by enhancing the GABA release, thereby supporting GABAergic mechanism.

Maximal electroshock-induced seizure model animals were divided as per the INH-induced seizure model. The positive control group animals were treated with phenytoin (20 mg/kg). After 30 min of treatments, the electroshock was induced in animals by passing the current of 150 mA for 0.2 second duration through auricular electrodes. The latency and incidence of tonic hind limb extension (THLE) and mortality rate was observed for 15 min. At 50 mg/kg dose of UASG exhibited significant ($P < 0.001$) reduction in THLE and 83.33% protection against mortality. Protection against THLE in MES-induced seizure also indicates the ability of the testing material to inhibit or prevent seizure discharge within the brain stem substrate [6]. UASG thus shows the anticonvulsant potential in the MES-induced seizures (Table 2).

Hence, the present study reveals that the ursolic acid stearoyl glucoside obtained from *Lantana camara* L. exhibited potential antiepileptic activity via facilitation of GABA transmission.

Conflict of Interest

The authors declare no conflict of interest.

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