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Shamshir khan
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi 110 062 India.

Bahar Ahmed
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi 110 062 India.

Habibullah Khalilullah
(a) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi 110 062 India.
(b) Department of Pharmaceutical Chemistry, Unaizah College of Pharmacy, Qassim University, Kingdom of Saudi Arabia.

Mubashir H Masoodi
Department of Pharmaceutical Sciences, University of Kashmir, Hazratbal, Srinagar, Jammu and Kashmir, India – 190006.

Correspondence:
Shamshir khan
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi 110 062 India.

Neuropharmacological activity of *Euphorbia hirta* and its isolated compound

Shamshir khan, Bahar Ahmed, Habibullah Khalilullah, Mubashir H Masoodi

ABSTRACT
Euphorbia hirta is a very popular herb amongst practitioners of traditional herbal medicine for its hypotensive, tonic, antipyretic, antiinflammatory, hypoglycemic and sedative activities. Moreover it is also used in different systems of medicine in the treatment of diarrhoea, bronchitis, skin diseases, fever, analgesic, gastrointestinal disorders, vomiting, wound healing, respiratory diseases and pulmonary disorders etc. Thus the aims of present study to assess the neuropharmacological activity of whole plant extract and characterized active constituents in rodent models of neurological disorders. The animals were treated with ethanolic extract, methanolic fraction and isolated compound; EH-1 (β-Stigmasterol glucoside), and anticonvulsant effect were evaluated against; Strychnine induced convulsion in mice; pentylenetetrazole (PTZ) induced seizure in mice; and maximal electroshock induced seizure in mice. The motor co-ordination, nootropic and anxiolytic activity was assessed in rats using rota rod and cook's pole climb apparatus and elevated plus maze respectively. The methanolic fraction has shown significant anticonvulsant, locomotor, nootropic and anxiolytic activity. However the isolated compound has not shown significant neuropharmacological activity. In conclusion the methanolic fraction and ethanolic extract (150 mg/kg p.o.) have shown significant neuropharmacological activities that could be the abundance of antioxidant constituents viz glycoside, flavonoids and phenolic compounds however isolated compound β-Stigmasterol glucoside did not shown significant effect.

Keywords: *Euphorbia hirta,* locomotor, anticonvulsant, nootropic, anxiolytic activity.

1. Introduction
Euphorbia hirta L. belongs to the family Euphorbiaceae, an herbaceous wild plant native to Australia, is now very common in all tropical countries and has been widely used in traditional medicine in Asia, the Middle East, Africa and the Caribbean, where 183 vernacular names have been recorded [1]. It is a small, erect or ascending annual herb reaching up to 50 cm, with hairy stems. The leaves are opposite, elliptical, oblong or oblong-lanceolate, with a faintly toothed margin and darker on the upper surface. The flowers are small, numerous and crowded together in dense cymes about 1 cm in diameter. The fruits are yellow, three-celled, hairy, keeled capsules, 1-2 mm in diameter, containing three brown, four-sided, angular, wrinkled seeds. The plant has been recommended for various therapeutic indications in traditional medicine, like hypotensive, tonic, antipyretic, antiinflammatory, hypoglycemic and sedative activities [2-11]. Chemical composition of *E. hirta* has been widely studied and a review has been previously published [12].
The plants of this genus *Euphorbia* contain many kinds of secondary metabolites which may be grouped into three main classes of compounds, namely terpenes (diterpenes, triterpenes and sesquiterpenes), phenolic derivatives (flavonoids, coumarins, acetophenones, lignans) and some alkaloids. More than 120 species of the genus have been studied for their chemical constituents, many of which have yielded several new classes of diterpenoid and triterpenoid skeleton. Some of the unusual diterpenoids are exemplified by euphorcinol isolated from *E. tirucalli* [13], enukokurin isolated from *E. lateriflora* [14], kansuiphorin A and B isolated from *E. kansui* [15].
Thus the present investigation was undertaken to determine the possible neuropharmacological effects like anticonvulsant, anxiolytic and antidepressant. The aerial parts of plant are well investigated for chemical information [16]. The chemical compound isolated from the plant include euphorbianin, leucocyanidol, camphol, quercitrin and quercitol [17, 18], gallic acid, myricitrin, 3,4-di-O-galloylquinic acid, 2,4,6-tri-O-galloyl-Dglucose, 1,2,3,4,6-penta-O-galloyl-β-D-glucose [19, 20], euphorbins A, B, C, D, E [21], β-amyrin, 24-methylenecycloartenol and β-Sitosterol [22].

2. Experimental

2.1 Materials and Methods

All chemicals and reagents were obtained from SD Fine-Chem Ltd., Mumbai and were of analytical reagents (AR) grade. Silica gel (60-120) mesh and silica gel-G were used for column chromatography and thin layer chromatography respectively. Melting point was determined on Perfit m.p. apparatus and was uncorrected. Infra-red (IR) spectra were recorded on Hitachi-270. ^{1}H NMR spectra were recorded on Bruker DRX-400 (400 MHz FT-NMR) using, $CDCl_3$ and DMSO-d_6 as solvent and TMS as internal standard. ^{13}C NMR spectra were recorded on DRX-400 (400 MHz FT-NMR) with TMS as internal standard in 5mm spinning tubes at 27 ^{o}C. Mass spectra (MS) were scanned by effecting Electron Impact (EI) ionization at 70 eV on a JEOL-JMS-DX 300 and FAB on JEOL SX 120/DA-6000 instrument equipped with direct inlet probe system.

2.2 Plant Material

The whole plant of *E. hirta* L. was collected from the Jamia Hamdard campus, New Delhi and was authenticated by the taxonomist Prof. Dr. M. P. Sharma, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi, India. The voucher specimen (EH-17) of the plant has been kept in the laboratory of Jamia Hamdard for future reference.

2.3 Isolation and characterization of compound

The plant material (3 kg) was dried and crushed to coarse powder and then successively extracted with methanol using cold percolation method till completely exhausted. The crude extract was dried under reduced pressure to get 435 gm of crude extract. Then it was fractionated with petroleum ether, chloroform and methanol to get mass of 85.0, 165.0, and 190.0 g respectively. 100 mg of methanol fraction was dissolved in little methanol and adsorbed on the silica gel (60-120 mesh) for the preparation of slurry. It was then dried, packed on the top of silica gel column packed in petroleum ether. The column was then eluted with petroleum ether, chloroform and methanol successively in the order of increasing polarity for the isolatation of compound.

2.4 Animals

Swiss albino mice, weighing between 25 and 30 g, were used for anticonvulsant activity. Albino wistar rats of either sex weighing 180-250 g were used for motor incoordination, nootropic and anxiolytic activity. The animals were kept in polypropylene cages under standard conditions (12-h light and 12-h dark cycles; 25 ±2 ˚C temperature) and had a free access to commercial pellet diet and water ad libitum. The protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Hamdard University (Proposal number: 461). All experiments were performed during the daytime and utmost care was taken to ensure that animals were treated in the most humane and ethically acceptable manner.

2.5 Drugs and treatment

PTZ and Strychnine was procured from Sigma (St Louis, MO, USA) and was used to obtain convulsion. Pentobarbitone was obtained as a gift sample from Ranbaxy Research Laboratory, Gurgaon, India. 30% polyethylene glycol 400 (PEG 400) were procured from S.D fine, India.

2.6 Anticonvulsant Activity

2.6.1 Strychnine-induced convulsion

Mice of either sex (n=6) were randomly distributed to control group (saline 0.9%w/v, 10 ml/kg, p.o.), Ethanolic freeze dried extract (Et Ext 50, 100 and 150 mg/kg, p.o.), freez dried methanolic fraction (MeOH Fr 100 mg/kg, p.o.), isolated compound (EH-1 20 mg/kg, p.o) groups. Strychnine hydrochloride (4 mg/kg, i.m.) was administered 30 min after treatment, and onset to tonic convulsion and number of mice showing tonic convulsion as well as mortality was recorded. Pentobarbitone 40 mg/kg, i.p. was used as a reference [23].

2.6.2 Pentylenetetrazole (PTZ)-induced convulsion

Pentylenetetrazole (PTZ) was used at a dose of 60 mg/kg intraperitoneally (i.p). The Swiss albino mice animals were divided into seven group having six mice in each group, treated with normal saline (0.9%w/v, 10 ml/kg, p.o) served as control, Ethanolic freeze dried extract (Et Ext 50, 100 and 150 mg/kg, p.o.), freeze dried methanolic fraction (MeOH Fr 100 mg/kg, p.o.), isolated compound (EH-1 20 mg/kg, p.o) groups. Thirty minutes later, seizures were induced by the i.p administration of 60 mg/kg of PTZ. The following parameters were observed during the first 30 min: severity of convulsions, percentage of death per group, percentage of animals which developed seizures per group, latency for death and latency to the first convulsion. The assessment was based on the method of Vohora et al., [24] and Fisher [25] which is based on the modification in the method of Snead [26] and Swinyard *et al.* [27].

2.6.3 Maximal Electroshock Seizure test (MES)

The MES is a model used for generalized tonic-clonic seizures. It is highly reproducible with consistent endpoints. The behavioural and electrographic seizures generated in this model are consistent with the human disorder [27]. In this method the maximal seizures were induced by the application of electrical current to the brain via ear clip electrodes with the help of instrument electroconvulsiometer (Hicon, India). The stimulus parameters for mice were 45 mA in a pulse of 60 Hz for 200 ms.

Swiss albino mice (female) were pre-screened 24 h before and divided into seven group having six mice in each group and treated with PEG 1ml/kg, (b.w) served as control, ethanolic freeze dried extract (Et Ext 50, 100 and 150 mg/kg, b.w), freez dried methanolic fraction (MeOH Fr 100 mg/kg, b.w), isolated compound (EH-1 20 mg/kg, b.w) and Phenytoin (PHT) 20 mg/kg, b.w. Test solution of all extracts and drugs was prepared in 30 % v/v polyethylene glycol 400 (PEG) and administered intraperitoneally (*i.p*) 30 minutes before the test. The abolition of hind limb tonic extensor of the seizure in half or more in the animals is defined as protection.

2.7 Motor coordination activity

Wistar albino rats were trained to maintain balance for 2 min on the rod rotating at the speed of 25 rpm. Only those rats which could balance themselves were selected for the study. Each rat was placed individually on the rota rod and the total number of falls within 2 min was noted, which was considered as the basal reading. Subsequently, the animals were divided into seven groups, each consisting of six animals. One hour following the administration of vehicle (control group) 2% Acacia suspension (10 ml/kg, p.o.), Ethanolic freeze dried extract (Et Ext 35, 70 and 105 mg/kg, p.o.), freeze dried methanolic fraction (MeOH Fr 70 mg/kg, p.o.), isolated compound (EH-1 20 mg/kg, p.o) and diazepam (2 mg/kg, p.o.,

positive control group), the rats were again placed on the rota rod and the number of falls per 2 min were recorded [28-30].

2.8 Nootropic activity using Cook's pole apparatus

The nootropic activity was assessed by using Cook's pole apparatus (Hichon, India), which uses the conditioned avoidance response as an index for evaluation of nootropic activity. The apparatus consisted of a sound proof experimental chamber, with a grid floor, which could be electrified and with a provision for a buzzer tone. A wooden pole, screwed onto the inner surface of the lid of the chamber acts as the shock free zone. In the assessment of nootropic activity, the stimulus provided was a foot shock of 6 mA given for a period of 10 s from the electrified grid floor. Rats were initially trained to escape the foot shock by climbing on to the pole, i.e. the shock free zone and only those rats, which could climb the pole and escape the foot shock were included in the study [31, 32].

The animals were divided into seven groups, each group consisting of six animals. The control group received 2% Acacia suspension (10 ml/kg, p.o.), Ethanolic freeze dried extract (Et Ext 35, 70 and 105 mg/kg, p.o.), freez dried methanolic fraction (MeOH Fr 70 mg/kg, p.o.), isolated compound (EH-1, 100 mg) and piracetam (positive control 100 mg/kg, p.o.) were administered to the rats for a period of 7 days. The notropic activity was evaluated at the end of 1st, 3rd and 7th day post treatment. On the day of evaluation, 1 hour post administration the acquisition trial (AT) was conducted and 24 h later the animals were subjected to the retention trial (RT). The acquisition trial consisted of 10 trial sessions interspersed with an interval of 30 s. During each trial the rats were allowed to explore the apparatus for 10 s, followed by a buzzer tone of 50 Hz (conditioned stimulus) for 10 s. This was followed by the foot shock for 10 s. The animal learns to associate the buzzer tone with the impeding foot shock and is capable of avoiding the foot shock on hearing the buzzer. The percent avoidance responses (AR) for the 10 trials were computed. RT comprising of 10 trials was carried out after 24 hr post-treatment and the number of AR in 10 trial sessions was computed.

2.9 Anxiolytic activity in rats using the elevated plus maze

Elevated plus maze is the simplest apparatus to study anxiolytic response of almost all types of anti-anxiety agents. The maze consisted of two opposite open arms (50 cm×10 cm), crossed with two enclosed arms of the same dimensions with walls 40 cm high. The arms were connected with a central square, 10 cm×10 cm to give the apparatus a plus sign appearance. The maze was elevated 70 cm above the floor in a dimly lit room [33]. Rodents have a natural aversion for high and open spaces and prefer enclosed arms, which have a burrow like ambience and therefore spend greater amount of time in the enclosed arm. When exposed to the novel maze alley, the animals experience an approach-avoidance conflict, which is stronger in the open arm as compared to the enclosed arms.

The animals were divided into seven groups, each group consisting of six animals. The control group received vehicle 2% Acacia suspension (10 ml/kg, p.o.), Ethanolic freeze dried extract (Et Ext 35, 70 and 105 mg/kg, p.o.), freez dried methanolic fraction (MeOH Fr 70 mg/kg, p.o.), isolated compound (EH-1; 20 mg/kg, p.o) and diazepam (2 mg/kg, p.o., positive control group) was administered to the different groups of animals. One hour after the administration of different extracts and drugs to respective groups, each rat was placed individually at the corner of an open arm and observed for a period of 5 min [34]. The animals were observed for; preference for open arm as first entry, number of open arm entries and duration of stay in the open arm. Increased exploratory activity in the open arm was an indication of anxiolytic activity.

2.10 Statistical analysis

The results were analysed by ANOVA followed by Dunnett's t test. Statistical analyses were performed using Graph pad Prism 3.0 (San Diego, CA, USA). P values <0.05 were considered as statistically significant.

3. Results and Discussion

3.1 Isolation of Constituent

Elution of column with $CHCl_3$-MeOH (1-2%) yielded compound **EH-1,** which was further recrystallised in $CHCl_3$-MeOH (1:1) to give the compound **EH-1** (400mg) as white crystal. The R_f value of isolated compound was 0.53 ($CHCl_3$-Pet. Ether, 6:4). Further the structure of the isolated compound was elucidated by IR, UV, NMR and mass spectroscopy (table 1, Figure 1).

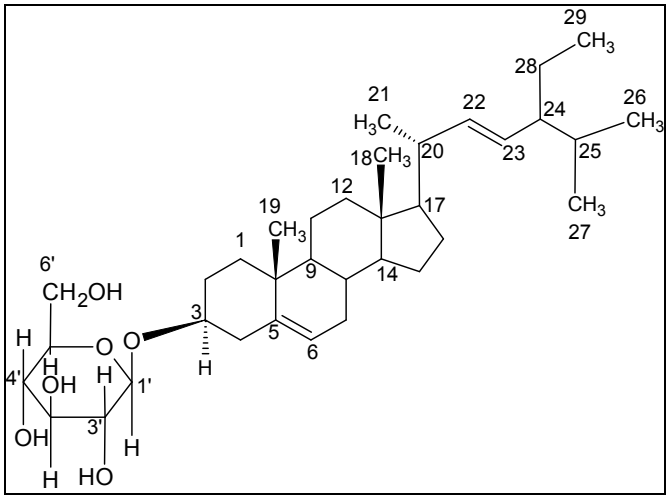

Fig 1: Chemical structure of β-Stigmasterol glucoside

Table 1: Compounds isolated from the whole plant of *E. hirta*.

Code	Compound Name	m.p. (°C)	Mol. Formula Mol. Wt.	IUPAC Name
EH-1	β-Stigmasterol glucoside	254-256 °C	$C_{35}H_{59}O_6$ 574	Stigmast-5-en-3-*O*-β-D-glucopyranoside

IR (KBr): ν_{max} 3433 (OH), 2960 (CH_3), 2850 (CH_2), 1463, 1062 (C-O), 810 (C=C), 728, 719 cm^{-1}
1D and 2D NMR ($CDCl_3$): Table 2
UV: λ_{max} 244, 204 (*sh*) nm.

EIMS (probe) 70 eV, *m/z* % (rel. int): 574 (M^+ $C_{35}H_{59}O_6$) (30), 301 (20), 367 (22), 255 (25), 255 (11), 177 (15), 163 (26), 159 (18).

Table 2: 1D and 2D NMR spectral data of β-Stigmasterol glucoside

Position	^{1}H NMR[a]	^{13}C- NMR / HMQC	^{1}H-^{1}H COSY	DEPT[b]	HMBC[c]	
					$2J_{CH}$	$3J_{CH}$
1a	1.18 *ddd* (16.0, 9.5, 5.3)	37.2 *t*	H-1b, H_2-2	CH_2	C-2, C-10	C-3, Me-19
1b	1.65 *ddd* (16.0, 8.5, 4.5)	-	H-1a, H_2-2	-	C-2, C-10	C-3, Me-19
2a	1.44 *ddd* (15.0, 8.5, 4.5)	31.6 *t*	H-2b, H-3, H_2-1	CH_2	C–1, C–3	C–10, C-5
2b	1.75 *m*	-	H-2a, H-3, H_2-1)	-	C–1, C–3	C–10
3α	3.52 *dddd* (5.2, 4.4, 5.6, 8.8) W1/2= 16 Hz	71.8 *d*	H_2-2, H_2-4	CH	C–2, C–4	C-5
4a	1.99 *dd* (11.5, 12.5)	42.2 *t*	H-4b, H-3	CH_2	C–3, C–5	C-6
4b	2.08 *ddd* (12.5, 6.5, 5.0)	-	H-4a, H-3	-	C–3, C–5	C-2
5	-	140.7 *s*	-	C	-	-
6	5.35 *t* (6.0)	121.7 *d*	H_2-7	CH	C-5, C-7	C-10, C-8, C-4
7a	1.67 *m*	33.9 *t*	H-7b, H-6	CH_2	C-6, C-8	C-5
7b	1.16 *m*	-	H-7a, H-8	-	C-6, C-8	C-9
8	1.12 *m*	31.9 *d*	H-7a, H-9	CH	C-9, C-7	C-6, C-10
9	0.78 *m*	50.1 *d*	H-8, H_2-11	CH	C-8, C-10, C-11	C-7, Me-19
10	-	36.1 *s*	-	C	-	-
11a	1.27 *ddd* (15.0, 9.5, 4.0)	21.1 *t*	H-9, H-11b, H_2-12	CH_2	C-9, C-12	C-8, C-13
11b	1.29 *ddd* (15.0, 6.5,5.0)	-	H-9, H-11a, H_2-12	-	C-9, C-12	C-13
12a	1.82 *m*	39.7 *t*	H_2-11	CH_2	C-11	C-13, Me-18, C-14
12b	1.84 *m*	-	H_2-11	-	-	Me-18, C-14
13	-	42.2 *s*	-	C	-	-
14	0.87 *m*	56.7 *d*	H-8, H_2-15	CH	C-8, C-15, C-13	C-16
15a	1.82 *m*	24.2 *t*	H_2-16, H-14, H-15a	CH_2	C-14, C-16	C-8, C-17
15b	1.84 *m*	-	H-14, H-15a, H_2-16	-	C-14, C-17	C-8
16a	0.94 *ddd* (13.0, 6.5, 3.5)	28.2 *t*	H-16b, H-17, H_2-15	CH_2	C-15, C-17	C-14
16b	1.84 *m*	-	H-16b, H-17,	-	C-15, C-17	C-13
17	2.08 *ddd* (9.0, 8.5)	56.1 *d*	H_2-16, H-20	CH	C-20, C-13	C-16
18	0.68 *s*	11.9 *q*	-	CH_3	C-13	C-12, C-17, C-14
19	1.0 *s*	19.3 *q*	-	CH_3	C-10	C-1, C-5, C-9

20	1.16 *m*	36.1 *d*	H-17, Me-21, H_2-22	CH	Me-21, C-17, C-22	C-23
21	0.91 *d* (5.2)	18.7 *q*	H-20	CH_3	C-20	C-17, C-22
22a	1.12 *m*	33.9 *t*	H-20, H_2-23, H-20b	CH_2	C-20, C-23	Me-21
22b	1.74 *m*	-	H-20, H_2-23	-	C-20	C-25, C-20, C-24
23a	0.90 *m*	26.09 *t*	H_2-22, H_2-24	CH_2	C-24, C-22	C-25, C-20
23b	0.93 *m*	-	H_2-22, H_2-24	-	C-24, C-22	C-25, C-20
24	0.78 *m*	45.8 *d*	H-25, H_2-23, H_2-28	CH	C-25, C-28	C-29
25	1.47 septet (W1/2=24.7)	29.1 *d*	H-24, Me-26, Me-27	CH	C-24, Me-26, Me-27	-
26	0.82 *d* (6.0)	19.7 *q*	H-25	CH_3	C-25	C-27
27	0.80 *d* (6.0	19.0 *q*	H-25	CH_3	C-25	C-26
28a	1.15 *ddd* (14.0, 10.5, 8.0)	23.0 *t*	H-24, Me-29	CH_2	C-24, C-29	C-25
28b	1.20 *ddd* (14.0, 7.5, 10.5)	-	H-24, Me-29	-	C-24, C-29	C-25
29	0.84 *d* (6.0)	11.8 *q*	H-28	CH_3	C-28	C-24

3.2 Strychnine-induced seizures

Ethanolic extract in graded dose as well as, methanolic fraction of ethanolic extract were showed a dose-related delay of the onset to tonic convulsion caused by strychnine. Even if it was unable to prevent convulsion and inhibition of mortality was also observed with 150 and MeOH 100 mg/kg. The ethanolic extract (150 mg) and MeOH 100 mg showed significant ($p< 0.05$) delay in onset of tonic convulsions. However, the isolated compound **EH-1** 40mg and Et Ext (50 and 100 mg) failed to delay convulsion (Table 3). Only treatment group Et Ext 150 mg and MeOH 100 mg showed anticonvulsant effect by delaying seizure produced by strychnine which indicates that it might produce its central depressant action as consequence of its glycinergic transmission, since strychnine antagonizes the inhibitory spinal cord and brainstem reflexes of glycine [35]. The anticonvulsant activity of methanolic extract could be due to its antioxidant and abundance of polyphenolic compounds which is incongruent with the earlier antioxidant findings of methanolic and aqueous extract of leaves, flowers and roots of *E. hirta* [36]. Although the exact antiepileptic mechanism of *E. hirta* observed in the present study is not clear. The experimental and clinical evidences suggest that inflammation and neurogenesis seem to be common factors contributing to the development of epilepsy [37]. The anti-inflammatory activity of E. hirta has been extensively reported in experimental studies [38]. Moreover, the anti-inflammatory effect *E. hirta* also contribute to the anticonvulsant activity as observed in the present study.

Table 3: Effect of ***Euphorbia hirta*** extract, fraction and their isolated compound on Strychnine induced seizure in mice.

S. No.	Treatment/ Group (mg/kg)	Onset of tonic convulsion (min.)	Mice showing convulsion	Mice Showing Mortality (within 24hr)
1.	Control	2.8 ± 0.52	6/6	6/6
2.	Et Ext 50mg	3.13 ± 0.67	6/6	5/6
3.	Et Ext 100mg	5.43 ± 0.74	6/6	6/6
4.	Et Ext 150mg	7.67 ± 3.86*	5/6	4/6
5.	MeOH Fr 100mg	7.5 ± 5.89*	4/6	3/6
6.	EH-1, 0mg	2.71 ± 0.79	6/6	6/6
7.	Pentobarbitone 40 mg	NC	2/6	1/6

Values are expressed as mean ± S.E.M.; n= number of animals in each group (n=6); NC=No convulsion;
* $p< 0.05$ when compared with control, significant by ANOVA followed by Dunnett's t-test.

3.3 PTZ-induced seizures

As shown in table 4, all doses of Et Ext (50, 100, 150 and MeOH Fr 100 mg increased the latency to the first convulsion and diminished the severity of convulsions. Only at doses of Et Ext 100, 150 and MeOH Fr 100 mg decreased the percentage of animals developing convulsions as well as decreased the number of deaths in these groups. The isolated compound, EH-1 40 mg did not show any significant effect on convulsion ($p > 0.05$). The present data indicates that PTZ at convulsant dose induce oxidative stress as reported by Obay *et al*., [39].

The anticonvulsant effect of *E. hirta* is due to its antioxidant constituents of the extract. These findings are in line with the previous report that hydroalcoholic extract of *Emblica officinalis* Gaertn shown antiepileptic, antioxidant and alleviation of cognitive impairment in PTZ treated rats [40].

3.4 Maximal Electroshock (MES)-induced seizure

In the MES-seizures test (Table 5), all doses of Et Ext (50, 100, 150 and MeOH Fr 100 mg increased the latency to the first convulsion and diminished the severity of convulsions. Only at doses of Et Ext 100, 150 and MeOH Fr 100 mg decreased the percentage of animals developing convulsions as well as decreased the number of deaths in these groups. The isolated compound, EH-1 40 mg did not show any significant effect on convulsion ($p > 0.05$). MES induced seizure can be

prevented either by drugs that inhibits voltage-dependent Na^+ channels or by drugs that block glutaminergic receptors such as felbamate. The anticonvulsant effect of E. hirta against MES induced seizure is due to presence of flavanoids and phenolic compounds [41]. Moreover, flavonoids and important class of natural compounds have demonstrated CNS activities such as affinity for $GABA_A$ receptors and anticonvulsant effect [42].

Table 4: Effect of ***Euphorbia hirta*** extract, fraction and their isolated compound on PTZ-induced seizure in mice.

S. No.	Treatment (mg/kg, p.o)	First convulsion latency (Min.)	Development of Convulsion (%)	No. of clonic convulsion (in 30 min.)	No. of death/ group (30 min.)
1.	Control	3.23 ± 0.81	100	5.67 ± 1.21	4/6
2.	Et Ext 50mg	4.8 ± 0.84	100	4.67 ± 1.21	3/6
3.	Et Ext 100mg	7.17 ± 0.84**	66.67	4.17 ± 1.17	2/6
4.	Et Ext 150mg	14.55 ± 1.49**	50	2.33 ± 0.87**	1/6
5.	MeOH Fr 100mg	15.28 ± 1.72**	50	2.5 ± 1.05**	2/6
6.	EH-1, 20mg	3.85 ± 0.831	100	5.5 ± 1.05	3/6
7.	VPA 300mg (i.p)	> 30	00	0.67 ± 0.82	0/6

Values are expressed as mean ± S.E.M.; n= number of animals in each group (n=6); VPA, Valproic acid.
** p<0.01, when compared with control (PTZ), Significant by ANOVA followed by Dunnett's t-test.

Table 5: Effect of ***Euphorbia hirta*** extract, fraction and their isolated compound on Maximal Electroshock (MES)-induced seizure in mice

S. No.	Treatment (mg/kg, i.p)	Protection from HLE (%)	Latency of HLE (Sec)	Duration of HLE (Sec)
1.	Control (PEG)	00	1.93± 0.22	35.98± 4.36
2.	Et Ext 50mg	33.33	5.63 ± 0.95	19.6 ± 3.04**
3.	Et Ext 100mg	50.0	4.21 ± 0.66	27.16 ± 4.11
4.	Et Ext 150mg	66.66	8.6 ± 1.11**	21.41 ± 2.2*
5.	MeOH Fr 100mg	83.33	16.08 ± 2.48**	11.26 ± 2.96**
6.	EH-1, 20mg	00	0.0 ± 0.0	0.0 ± 0.0**
7.	PHT 20mg	100	No HLE	No HLE

Values are expressed as mean ± S.E.M.; n= number of animals in each group (n=6); HLE, Hind limb extention; PHT, Phenytoin.
* p<0.05, ** p<0.01, when compared with control (PTZ), Significant by ANOVA followed by Dunnett's t-test.

3.5 Motor in coordination (muscle relaxant activity)

There was no increase in the number of falls within 2 min; after treatment of the animals with vehicle 2% Acacia suspension, Et Ext (35, 70 and 105 mg), MeOH Fr 70 mg, isolated compound EH-1 20 mg. However, the diazepam treated group showed significant ($p < 0.01$) increase in the number of falls (12.67) as compared to the control (Table 6). In our experimental findings, the extracts did not demonstrate any effect on the muscle coordination, as indicated by the findings with respect to the rota rod model.

Table 6: Effect of extracts, fraction as well as isolated compound of *Euphorbia hirta* and diazepam on muscle-relaxant activity in rats, using rota rod apparatus

S. No.	Treatment/ Group (mg/kg, p.o)	Number of falls in 2 min	
		Basal Reading	After Treatment
1.	Control	6.33 ± 0.82	5 ± 1.55
2.	Et Ext 50mg	6.83 ± 1.17	5.83 ± 1.17
3.	Et Ext 100mg	5 ± 0.89	6 ± 1.26
4.	Et Ext 150mg	4.83 ± 1.47	6 ± 1.26
5.	MeOH Fr 100mg	6.5 ± 0.84	4.17 ± 1.72
6.	EH-1, 20mg	4 ± 1.41	4 ± 1.41
7.	Diazepam 2mg	5.33 ± 1.21	12.67 ± 2.81**

Values are expressed as mean ± S.E.M.; n= number of animals in each group (n=6).
** p< 0.01 when compared with control (PTZ), Significant by ANOVA followed by Dunnett's t-test.

3.6 Nootropic activity using Cook's pole apparatus

A single day treatment did not produce an increase in avoidance responses (AR) in the acquisition and retention trials and also there is no significant increase in AR in the acquisition trial (AT) on day three. However, on day three the MeOH Fr 70mg and piracetam treated groups demonstrated an significant ($P < 0.01$) increase in the number of AR during retention trial (RT) as compared to the control group. Hence, the drug administration was further increased to 7 days and the AT was performed on the end of the day of drug administration and 24 h later the RT was conducted. Pretreatment with Et Ext 70 mg for 7 days have significantly (P<0.05) increased the avoidance response in acquisition (41.70%) and in retention (46.7%) trial as compared to control

group. The treatment group, Et Ext 105 mg, MeOH Fr 70 mg and standard drug Piracetam 100 mg also produced significant ($P < 0.001$) increase in the number of AR as compared to the control; 46.7, 55, 66.7% and 60, 63.3, 75% AR in the acquisition and retention trials respectively. The Et Ext 35 mg and isolated compound EH-1, 100mg when administered for 7 days, failed to produce a significant increase in the AR; 28.3 and 40% ($P > 0.05$) in the acquisition trial (AT); 30.0 and 36.7% ($P > 0.05$) in the retention trial (RT) as compared to the 26.7 and 28.3% of control animals in AT and RT, respectively (Table 7).

Our findings indicated only group treated with Et Ext 105mg and MeOH 70 mg demonstrated a significant improvement in the acquisition and retention of memory of the learned task as was seen by the increase in the percent avoidance responses, thus demonstrating nootropic activity. This facilitatory effect on learning and memory was observed only after 7 days treatment. This probably may be attributed to the involvement of neurotransmitters since the building of memory is augmented only when the levels of neurotransmitters are attenuated on repeated administration of the extracts. There is ample evidence demonstrating that the central cholinergic system, serotonergic transmission and noradrenaline function play a vital role in the cognitive function of the brain [43-45]. However, in our experimental investigations neither Et Ext (35 mg) nor the isolated compound EH-1 demonstrated any effect on learning and memory. This could be due to the difference in the nature of the constituents.

Table 7: Effect of extracts of *Euphorbia hirta* and piracetam on nootropic activity in rats when administered for 3 and 7 days using Cook's pole apparatus

S. No.	Treatment Group (mg/kg, p. o)	Percentage avoidance response in AT (mean ± S.E.M)		Percentage avoidance response in RT (mean ± S.E.M)	
		Day 3	Day 7	Day 3	Day 7
1.	Control (10 ml/kg)	30 ± 8.94	26.67 ± 5.16	31.67 ± 7.53	28.33 ± 7.53
2.	Et Ext 35	30 ± 6.32	28.33 ± 7.53	33.33 ± 12.11	40 ± 12.65
3.	Et Ext 70	33.33 ± 10.33	41.67 ± 7.53*	40 ± 8.95	46.67 ± 10.33*
4.	Et Ext 105	35 ± 10.49	46.67 ± 12.11**	48.33 ± 11.69	60 ± 8.94**
5.	MeOH Fr 70	36.67 ± 12.11	55 ± 10.49**	56.67 ± 12.11**	63.33 ± 12.11**
6.	EH-1, 100	31.67 ± 7.53	30 ± 8.94	33.33 ± 10.33	36.67 ± 12.11
7.	Piracetam-100	40 ± 8.94	66.67 ± 8.16**	56 ± 13.42**	75 ± 10.49**

Values are expressed as mean ± S.E.M.; n= number of animals in each group (n=6); AT, acquisition trial; RT, retention trial.
* P < 0.05, ** P < 0.01 when compared with control, significant by one-way ANOVA followed by Dunnett's test.

3.7 Anxiolytic activity using elevated plus maze

When the animals were placed on the maze, they showed a preference for the enclosed (dark) arms and showed anxiety and fear like movements characterized by immobility, freezing and defecation on entering the open arms. The treatment group, Et Ext 105 mg and MeOH Fr 70 mg showed significant increase ($p < 0.05$) in no. of open arm entries 4.83 and 4.33 respectively. Whereas, only treatment group, Et Ext 105 showed significant increase ($p < 0.01$) in time spent in open arm (153 sec.) as well as % first preference open arm entry (83.33%) when compared to the control group.

Also the group Et Ext 105 mg and Diazepam 2 mg more significantly increased ($P < 0.01$) in the percent preference for open arm (83.33 and 83.33%), the number of entries (4.83 and 5.33) as well as the duration of stay in the open arms (153 and 161.33), indicating anxiolytic activity. However, the group MeOH Fr and EH-1 20mg failed to produce any significant effect (Table 8).

Table 8: Effect of extracts of ***Euphorbia hirta***, their isolated compounds and diazepam on anxiolytic activity in rats using elevated plus maze.

Treatment Group (mg/kg, p. o)	Preference (%) Open Arm	Time Spent (s) in Open Arm Mean ± S.E.M	No. of entries in Open Arm Mean ± S.E.M
Control (10ml/kg)	33.33	50.17 ± 23.36	1.5 ± 0.56
Et Ext 35 mg	50	89.83 ± 46.04	2 ± 0.68
Et Ext 70 mg	66.67	131.33 ± 48.29*	2.33 ± 0.76
Et Ext 105 mg	83.33	153 ± 67.01**	4.83 ± 1.01*
MeOH Fr 70 mg	33.33	107.5 ± 30.51	4.33 ± .92*
EH-1, 20 mg	50	85 ± 68.97	1.83 ± 0.65
Diazepam 2 mg	83.33	161.33 ± 60.29**	5.33 ± 0.42**

Values are expressed as mean ± S.E.M.; n= number of animals in each group (n=6).
* P < 0.05, ** P < 0.01 when compared with control, significant by one-way ANOVA followed by Dunnett's test.

The investigation showed that the extract Et Ext 105mg and Diazepam 2mg produced significant change in the exploratory activity of the rats in the elevated plus maze model which may be because of some anxiolytic compounds in extract mixture and thus decreasing anxiety, increase the open arm exploration time as well as the number of entries into the open arm. The isolated compounds, and lower dose of Et Ext 35 mg as well as MeOH Fr of the plant failed to demonstrate any such effect in the rats and hence we can conclude that Et Ext have anxiolytic activity at higher dose 70 and 105mg. It has been also noted is that recently the plus maze model is also being used to study learning and memory processes in rodents. The

impairment of learning and memory induced by scopolamine, an anti-cholinergic agent, is reflected by prolonged transfer latency from the open arm to the closed arm [46]. With respect to our findings, in contrast to that of diazepam, lower dose of the extracts and MeOH fr did not cause an increase in the number of entries into the open arm. It could thus also be inferred that the rats retain the memory of the aversive quality of the open arm and this could probably be considered a significant finding with respect to the plant extracts.

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6. References:

1. Lanhers MC. Contribution a létude ethnopharmacologique et étude pharmacologique d'*Euphorbia hirta* L.: propriétés psychotropes, analgesiques, anti-pyretiques et anti-inflammatoires. Thèse de doctorat de l'université de Metz, mention pharmacognosie, Centre des Sciences de l'Environnement, 1988, 629.
2. Watt JM, Breyer-Brandwijk MG. The Medicinal and Poisonous Plants of Southern and Eastern Africa 2nd Edn. E. and S. Livingstone, England, 1962; 1:408-411.
3. Martin M, Ridet J, Chartol A, Biot J, Porte L, Bezon A. Action therapeutique de l'extrait *d'Euphorbia hirta* dam l'amibiase intestinale. A propos de 150 observations. M & detine Tropicale 1964; 24(31):250- 261.
4. Ridet J, Chartol A. Les proprietes antidysenteriques de l'*Euphorbia hirta* Medecine Tropicale 1964; 24(21):119-143.
5. Steinmetz EF. *Euphorbia piluliferae* summitates. Quarterly Journal of Crude Drug Research. 1964; 4(21):548-551.
6. De-Saqui-Sannes G. Etude chimique de polyphenols naturels. Donnees structurales et pharmacologiques des principes actifs d'*Euphorbia hirta* L. (Euphorbiacees) These d'etat de docteur en pharmacie, Universite Paul Sabatier (UER des Sciences pharmaceutiques), Toulouse, 1971, 224.
7. Poulet E. Description et usage dune plante herbacee de Haute-Volta *Euphorbia hirta*. Notes et Documents Voltafques 1972; 6(1):25-30.
8. Zipcy E. Essai sur l' ethnopharmacologie du Cumeroun. These d'etat de docteur en pharmacie, Faculte de Pharmacie, Marseille. 1975, 119-121.
9. Ndir O, Pousset JL. Plantes medicinales africaines VIII Contribution a l'etude pharmacologique et chimique *d'Euphorbia hirta* L. Mddecine d'Afrique Noire. 1982; 29(7):503-518.
10. Dalil M. Essai therapeutique dun decocte lyophilise'd' *Euphorbia hirta* L. (mbau dans le traitement ambulutoire de l'amibiase intestinale. These detat de docteur en pharmacie. University de Dakar, Faculte de Medecine et de Pharmacie. 1984, 70.
11. Adjanohoun EJ, Ahyi AM, Ake Assi L, Akpagana K, Chibon P, El-Hadji A *et al*. Medicine traditionnelle and pharmacope'e. Contribution aux etudes ethnobotaniques et, floristiques au Togo. ACCT, Paris. 1986, 157.
12. Lanhers MC, Fleurentin J, Mortier F, Pousset JL, Pelt JM. Mini-review: composition chimique de *Euphorbia hirta* L. Al Biruniya 1987; 3(2):121-136.
13. Khan AQ, Kazmi SN, Ahmed Z, Malik A. Euphorcinol A new pentacyclic triterpene from *Euphorbia tirucalli.* Planta Med 1989; 55(3):290-291.
14. Connolly JD, Hill RA. Triterpenoids. Nat Prod Rep 1989; 6(5):475-501.
15. Wu TS, Lin YM, Haruna M, Pan DJ, Shingu T, Chen YP *et al.* Antitumor agents 119 Kansuiphorins A and B, two novel antileukemic diterpene esters from *Euphorbia kansui*. J Nat Prod 1991; 54(3):23-829.
16. Gupta DR. A chemical examination of *Euphorbia hirta* Linn. Bull Chem Soc Jpn 1966; 39(11):2532-2534.
17. Blanc P, Bertrand PG. De saqui-sannes Flavonoids of *Euphorbia hirta*. Plants Med Phytotherap 1972; 6:106-109.
18. Aquil M, Khan IZ. Euphorbianin a new glycoside from *Euphorbia hirta* Linn. Global J Pure Applied Sci 1999; 5(3):38.
19. Chen L. Polyphenols from leaves of *Euphorbia hirta* L. Zhongguo Zhong Yao Za Zhi 1991; 16(1):38-39.
20. Yoshida T, Chen L, Shingu T, Okuda T. Tannins and related polyphenols of euphorbiaceous plants IV. Euphorbins A and B, novel dimeric dehydroellagitannins from *Euphorbia hirta* L. Chemical and Pharmaceutical bulletin. 1988; 36(8):2940-2949.
21. Martínez-Vázquez M, Ramírez Apan TO, Lazcano ME, Bye R. Anti-inflammatory compounds from the n-hexane extract of euphorbia hirta, Revista de la Sociedad Química de México 1999; 43(3-4):103-105.
22. Yemitana OK, Salahdeen HM. Neurosedative and muscle relaxant activities of Aqueous extract of *Bryophyllum pinnatum.* Fitoterapia 2005; 76:187-193.
23. Vohora D, Pal SN, Pillai KK. Thioperamide a selective histamine H3 receptor antagonist protects against PTZ-induced seizures in mice. Life Sci 2000; 66:297-301.
24. Fisher RS. Animal models of the epilepsies. Brain Res Rev 1989; 14(3):245-278.
25. Snead OC. Pharmacological models of generalized absence seizures in rodents. J Neural Transm Suppl 1992; 35:7-19.
26. Swinyard EA, Woodhead JH, White HS, Franklin MR. General principles: experimental selection, quantification, and evaluation of anticonvulsants. In: Antiepileptic Drugs, Third Edition Levy, R.H., Mattson, R.H., Melrum, B., Penry, J.K., Dreifuss, F.E., (Ed), New York, Raven Press, 1989, 85-102.
27. Kulkarni SK, Joseph P. Psychopharmacological profile of Siotone granules a herbal preparation. Indian Drugs 1997; 35:536-545.
28. Turner RA. Screening Methods in Pharmacology. Academic Press, New York, 1965.

29. Kulkarni SK. Handbook of Experimental Pharmacology, (Ed. 3), Vallabh Prakashan, New Delhi, 1999.
30. Cook WJ, Weidley E. Behavioral effects of some psychopharmacological agents. Annals of the New York Academy of Sciences 1957; 66:740-752.
31. Reddy DS. Assessment of nootropic and amnestic activity of centrally acting agents. Indian J Pharmacol 1997; 29(4):208–221.
32. Jaiswal AK, Bhattacharya SK. Effects of Shilajit on memory, anxiety and brain monoamines in rats. Indian J Pharmacol 1992; 24(1):12-17.
33. Kulkarni SK, Verma A. Protective effect of BR-16A (Mentat), a herbal preparation on alcohol abstinence induced anxiety and convulsions. Indian J Exp Biol 1993; 31(5):435-439.
34. Biggio G, Cibin M, Diana M, Fadda F, Ferrara SB, Galimberti L *et al.* Suppression of voluntary alcohol intake in rats and alcoholics by gamma-hydroxybutyric acid: a non-GABAergicmechanism. Adv. Biochem Psychopharmacol 1992; 47:281-288.
35. Abu AB, Zuraini Z, Lacimanan YL. Antioxidant activity and phytochemical screening of the methanolic extracts of *Euphorbia hirta* L. Asian Pacific J Trop Med 2011; 4(5):386-390.
36. Okamoto OK, Janjoppi L, Bonone FM, Pansani AP, da Silva AV, Scorza FA *et al.* Whole transcriptome analysis of the hippocampus toward a molecular portrait of epileptogenesis. BMC Genomics 2010; 11:230-242.
37. Lanhers MC, Fleurentin J, Cabalion P, Dorfman P, Pelt JM, Dorfman P *et al.* Analgesic, antipyretic and anti-inflammatory properties of *Euphorbia hirta.* Planta Medica 1991; 7(3):225-231.
38. Obay BD, Tasdemir E, Tumer C, Bilgin H, Atmaca M. Dose dependent effects of ghrelin on pentylenetetrazole-induced oxidative stress in a rat seizure model. Peptides 2008; 29(3):448-455.
39. Golechha M, Bhatia J, Arya DS. Hydroalcoholic extract of *Emblica officinalis* Gaertn. Affords protection against PTZ-induced seizure oxidative stress and cognitive impairment in rats. 2010; 48(5):474-478.
40. Wu Y, Qu W, Geng D, Liang JY, Luo YL. Phenols and flavonoids from aerial parts of *Euphorbia hirta*. Chin J Nat Med 2012; 10(1):40-42.
41. Huen M, Leung J, Ng W, Lui W, Chan M, Tze-Fei Wong J *et al.* 5, 7-Dihydroxy-6-methoxy flavone, a benzodiazepine site ligand isolated from *Scutellaria baicalensis* Georgi, with selective antagonistic properties. J Biochem Pharmacol 2003; 66:125-132.
42. Hollander E, Mohs RC, Davis KL. Cholinergic approaches to the treatment of Alzheimer's disease. British Medical Bulletin 1986; 42(1):97–100.
43. Ogren SO. Biology of Serotonergic Transmission. John Wiley and Sons Chichester 1982; 317.
44. Nalini K, Karanth KS, Rao A, Aroor AR. Effect of *Celastrous paniculatus* on passive avoidance performance and biogenic amine turnover in albino rats. J Ethnopharmacol 1995; 47(2):101–108.
45. Iyer MR, Pal SC, Kasture VS, Kasture SB. Effect of *Lawsonia inermis* on memory and behavior mediated via monoamine neurotransmitters. Indian J Pharmacol 1998; 30(3):181–185.