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DEVELOPMENT OF DIETHYL MALEATE SENSITIZED 6HZ PSYCHOMOTOR SEIZURE MODEL IN MICE.

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Abstract: Purpose: Development of diethyl maleate (DEM) sensitized 6Hz psychomotor seizure model in mice. Methods: Psychomotor seizures were induced via corneal stimulation (6 Hz, 32mA or 44 mA intensity for 3s duration). The animals were observed for behavioral observations for 120s duration - Stun/stupor, twitching of vibrissae and straub tail. Markers of oxidative stress (reduced glutathione [GSH] and malondialdehyde [MDA]) were estimated in mice brain. Results: In DEM sensitized 6Hz psychomotor seizure (44mA for 3sec) model duration of stun/stupor, twitching of vibrissae and straub tail were more & level of oxidative stress were more as compared to DEM sensitized 6Hz psychomotor seizure (32mA for 3sec) group. Conclusion: DEM sensitized 6Hz psychomotor seizure (44mA for 3sec) can be used as a model for evaluation of drugs used in pharmaco-resistant epilepsy.

Keywords: Diethyl maleate (DEM), 6Hz psychomotor seizure, Reduced glutathione [GSH], Malondialdehyde [MDA]

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INTRODUCTION

Several evidences supports the role of oxidative stress in refractory epilepsy[1,2,3,4]. Drug-resistant seizures exhibit enhanced brain expression of P-glycoprotein[5,6,7,8]. Experimental evidence supports the involvement of oxidative stress induced by glutathione depletion involved in upregulation of P-glycoprotein at the blood– brain barrier in rats[9,10].

Several studies have shown that diethyl maleate (DEM) is a glutathione depleting agent and induces oxidative stress[9,11]. Glutathione depletion involved in upregulation of P-glycoprotein expression at the blood-brain barrier in rats [9,10].

6Hz psychomotor seizure model is an acute model for pharmacoresistant epilepsy[12]. Stun/stupor, twitching of vibrissae and straub tail are characteristic parameters in 6Hz psychomotor seizure model, resulting in development of resistant epilepsy[12]. As reported earlier that even acute electroshock treatment produces an increase in the lipid peroxidation and thus increases oxidative damage[13,14].

The above knowledge was utilized in the study for the development of DEM sensitized 6Hz psychomotor seizure model.

MATERIALS AND METHODS

Animals

Male Swiss strain albino mice weighing between 18–25 g procured from central animal house facility of Jamia Hamdard, were used. The animals were housed in polypropylene cages, maintained at 21±1^{0}C and 12 h light/dark cycle. After 5 days of acclimatization to laboratory conditions, the animals were randomly assigned to different experimental groups. All experiments were performed during the daytime on healthy animals. The experimental protocol and procedures listed were approved by Jamia Hamdard Animal Ethics Committee (JHAEC project no. 1107).

Drugs and chemicals

DEM and Olive oil were procured from Sigma-aldrich, USA. DEM was dissolved in olive oil and was administered at a dose of 844mg/kg, i.p. 2 hours before 6 Hz stimulation[9,11]. Drug solutions were freshly prepared on each day of experimentation.

6 Hz psychomotor seizure test

Psychomotor seizures were induced via corneal stimulation (6 Hz, 32mA or 44 mA intensity for 3s duration) using a electroconvulsiometer (Ugo Basile, Itlay)[12]. Saline solution was applied to the corneas of the animal before the stimulation. Animals were restrained during the stimulation and were released immediately following it. The animals were observed for behavioral observations for 120s duration for each animal in a group. Stun/stupor, twitching of vibrissae and straub tail are characteristic behavioral parameters in 6Hz psychomotor seizure model, resulting in development of resistant epilepsy[12]. Protection against the seizures was regarded as the endpoint of the study. The animal was considered protected if it resumed the normal behavior within 10s after the stimulation[15].

Tissue homogenate preparation

The animals were sacrificed by cervical decapitation under light anesthesia (diethyl ether) on the 3rd day, immediately after behavioral assessments. Then whole brain was carefully removed, tissue homogenate (10% w/v) was prepared in 0.1 M KCl solution and homogenized in an ice bath. The homogenate was centrifuged at 3000 rpm for 10 minutes at 4^0C and the resultant cloudy supernatant liquid was used for estimation of protein, brain malondialdehyde (MDA), reduced glutathione (GSH) level.

Biochemical Estimations

Estimation of Brain Reduced Glutathione (GSH)

GSH estimation in brain homogenate was measured according to the Ellman method[16]. This method is based on the development of a yellow color when 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) is added to the compound containing the sulfhydryl groups. The brain homogenate (0.5ml) was mixed with 0.5ml TCA (6%) and then centrifuged (cold-2 to 4^0C) at 3000 rpm for 10 minutes. After that, 0.2 ml of supernatant was mixed with 4.7 ml of 0.1 M phosphate buffer (pH 8) and 0.1ml DTNB (0.01 M). The absorbance was read within 5 minutes of the addition of DTNB at 412 nm spectrophotometrically against a reagent blank with no homogenate. [In blank, 0.5 ml KCl of 0.15 M was used instead of brain homogenate]. The amount of GSH in the tissue expressed as µg/mg of protein. Tissue protein was estimated using Lowry method of protein assay[17].

Estimation of brain malondialdehyde (MDA)

Malondialdehyde, indicator of lipid peroxidation was estimated as described by Ohkawa et al.[18] The reaction mixture consisted of 0.2 ml of 8.1 % sodium lauryl sulphate, 1.5 ml of 20 % acetic acid (pH-3.5) and 1.5 ml of 0.8 % aqueous solution of thio-barbituric acid (TBA) was added to the 0.2 ml of processed brain homogenate. The mixture was made

up to 4.0 ml with distilled water and heated at 95˚C for 60 minutes. After cooling with tap water, 5 ml of n-butanol and pyridine (15:1 v/v) and 1 ml of distilled water was added and centrifuged. The organic layer was separated out and its absorbance was measured at 532 nm using a UV-Visible spectrophotometer against blank with no homogenate. [In blank, 0.2ml KCl of 0.15 M was used instead of brain homogenate]. The amount of MDA in the tissue was expressed as nmole/ mg of protein. Tissue protein was estimated using Lowry method of protein assay[17].

Statistical analysis

The data were expressed as Mean ± S.D. and statistically analyzed using one way ANOVA followed by Dunnet 't' test and one way ANOVA followed by Tukey-Kramer's Multiple comparison test, $p<0.05$ was considered significant.

RESULTS

Effect of DEM sensitized 6Hz psychomotor seizure on duration of stun/stupor, twitching of vibrissae and straub tail in mice.

Control (olive oil – 10 ml/kg, i.p.) group did not receive 6Hz stimulation. No psychomotor behavioral parameters observed viz. no stun/stupor, no twitching of vibrissae and no straub tail.

In DEM (844 mg/kg, i.p.) group mice showed a siginificant stun/stupor for 44 ± 16.8 seconds, $p< 0.01$ when compared with control group (one–way ANOVA followed by Dunnett's t-test). However non-significant twitching of vibrissae was observed for 4.6±2.8 sec , $p > 0.05$ when compared with control group (one–way ANOVA followed by Dunnett's t-test). Whereas no straub tail was observed.

DEM sensitized 6Hz psychomotor seizure (32mA for 3sec) group showed a significant stun/stupor for 59.6 ± 19.3 seconds, a significant twitching of vibrissae for 36.7±15.9 sec and a significant straub tail for 31.5 ± 14.2 sec was observed, $p< 0.01$ when compared with control group (one–way ANOVA followed by Dunnett's t-test).

DEM sensitized 6Hz psychomotor seizure (44mA for 3sec) group showed a significant stun/stupor for 62.5± 22.8 seconds, a significant twitching of vibrissae for 42.9 ± 16.2 sec and a significant straub tail for 38.2± 18.4 sec was observed, $p< 0.01$ when compared with control group (one–way ANOVA followed by Dunnett's t-test).

Duration of stun/stupor, twitching of vibrissae and straub tail were more in DEM sensitized 6Hz psychomotor seizure (44mA for 3sec) group as when compared with DEM sensitized 6Hz psychomotor seizure (32mA for 3sec) group.

The results are summarized in Table 1 and Figure 1

Effect of DEM sensitized 6Hz psychomotor seizure on brain malondialdehyde (MDA) level and glutathione (GSH) level in mice.

The mean brain MDA level for mice belonging to Control (olive oil – 10ml/kg i.p.) group was found out to be 5.98707 nmole/mg of protein.

A significant increase (10.73779 nmole/mg of protein) in brain MDA level was found in mice treated with DEM. $p < 0.01$ when compared with control group (one–way ANOVA followed by Dunnett's t-test).

A significant increase (12.14546 nmole/mg of protein) in brain MDA level was found in mice belonging to group III (DEM + 6Hz 32mA). $p < 0.01$ when compared with control group (one–way ANOVA followed by Dunnett's t-test).

A significant increase (14.57347 nmole/mg of protein) in brain MDA level was found in mice belonging to group IV (DEM + 6Hz 44mA). $p < 0.01$ when compared with control group (one–way ANOVA followed by Dunnett's t-test).

The mean brain glutathione (GSH**)** level for mice belonging to Control (olive oil 10ml/kg i.p.) group was found out to be 5.85898 µg/mg of protein.

A significant decrease (4.27512 µg/mg of protein) in brain GSH level [GSH level decreased by 27.03%] was found in mice treated with DEM. $p < 0.01$ when compared with control group (one –way ANOVA followed by Dunnett's t-test).

A significant decrease (3.48124 µg/mg of protein) in brain GSH level [GSH level decreased by 40.58%] was found in mice belonging to group III (DEM + 6Hz 32mA). $p < 0.01$ when compared with control group (one –way ANOVA followed by Dunnett's t-test).

A significant decrease (3.189515 µg/mg of protein) in brain GSH level [GSH level decreased by 45.56%] was found in mice belonging to group IV (DEM + 6Hz 44mA). $p < 0.01$ when compared with control group (one –way ANOVA followed by Dunnett's t-test).

The results are summarized in Table 2 and Figure 2

DISCUSSION

The present study was conducted with a view to develop a pharmacoresistant epilepsy model in mice.

DEM at a dose of 844 mg/kg, i.p., produced stun/stupor position; however twitching of vibrissae and straub tail were non-significant. Biochemical estimations reflected a significant increase in brain malondialdehyde (MDA) levels and significant decrease in brain glutathione (GSH) level [GSH level decreased by 27.03%] as when compared with control group (olive oil – 10ml/kg, i.p.). Our results are in agreement with the results of **Wu J et al. (2009)**[9] and **Agarwal R et al. (1999)**[11]. The present results confirm that DEM a glutathione depleting agent that causes oxidative stress in mice brain.

DEM sensitized 6Hz psychomotor seizure (32mA for 3sec) model induced via corneal stimulation showed stun/stupor, twitching of vibrissae and straub tail which are characteristic parameters in 6Hz psychomotor seizure model in mice (**Barton et al. 2001**)[12]. Biochemical estimations reflected a significant increase in brain malondialdehyde (MDA) levels and significant decrease in brain glutathione (GSH) level [GSH level decreased by 40.58%] as when compared with control group (olive oil –10ml/kg, i.p.). As reported earlier that electroshock treatment produces an increase in the lipid peroxidation and thus increases oxidative damage[13,14]. DEM induces oxidative stress**;** hence in DEM sensitized 6Hz psychomotor seizure (32mA for 3sec) model produced more oxidative damage because GSH level decreased by 40.58%] as when compared with DEM treated groups.

DEM sensitized 6Hz psychomotor seizure (44mA for 3sec) induced via corneal stimulation produced all characteristic behavioral parameters viz. stun/stupor, twitching of vibrissae and straub tail; duration period was more than DEM sensitized 6Hz psychomotor seizure (32mA for 3sec) group. Biochemical estimations reflected more oxidative stress in brain as when compared with DEM sensitized 6Hz psychomotor seizure (32mA for 3sec) group.

DEM sensitized 6Hz psychomotor seizure (44mA for 3sec) model was being selected for our study as no mortality was observed and duration of stun/stupor and straub tail was more & level of oxidative stress was more as compared to DEM sensitized 6Hz psychomotor seizure (32mA for 3sec) group.

CONCLUSIONS

DEM sensitized 6Hz psychomotor seizure (44mA for 3sec) can be used as a model for evaluation of drugs used in pharmacoresistant epilepsy.

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CONFLICT OF INTEREST

The authors state no conflicts of interest.

Table 1: Effect of DEM sensitized 6Hz psychomotor seizure on duration of stun/stupor, twitching of vibrissae and straub tail in mice.

Groups (n=5)	Treatment	Duration of stun/stupor, twitching of vibrissae and straub tail (secs)		
		Stun/stupor	Twitching of vibrissae	Straub tail
I	Control- olive oil (10ml/kg i.p.)	0	0	0
II	DEM	44 ± 16.8 *	4.6±2.8 #	0 #
III	DEM + 6Hz (32mA)	59.6 ± 19.3*	36.7 ±15.9 *	31.5 ± 14.2 *
IV	DEM + 6Hz (44mA)	62.5 ± 22.8*	42.9 ± 16.2 *	38.2 ± 18.4 *

DEM = Diethyl maleate (844 mg/kg, i.p. - single dose) ; n = Number of animals in a group.

Values are expressed as Mean ± SD

* denotes $p < 0.01$ when compared with control group (one –way ANOVA followed by Dunnett's t-test). [significant].

# denotes $p > 0.05$ when compared with control group (one –way ANOVA followed by Dunnett's t-test). [Not significant].

Table 2: Effect of DEM sensitized 6Hz psychomotor seizure on brain malondialdehyde (MDA) level and glutathione (GSH) level in mice.

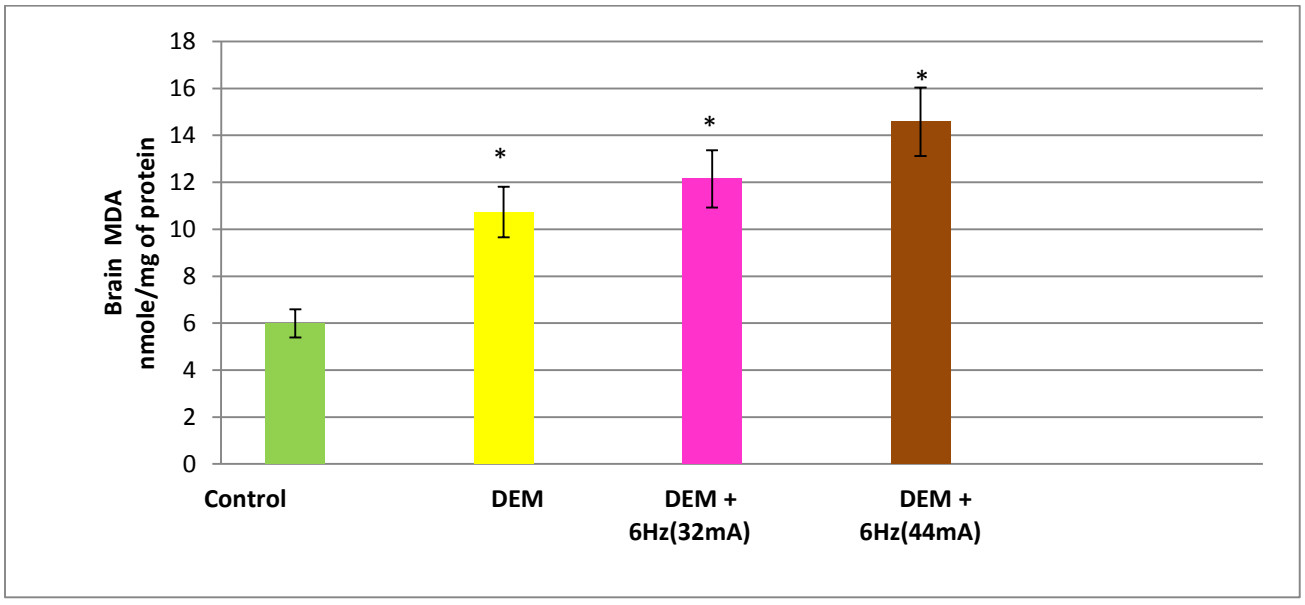
Groups (n=5)	Treatment	Brain MDA (nmole / mg protein)	Brain GSH (µg / mg protein)
I	Control (olive oil 10ml/kg,i.p.)	5.98707 ± 0.1405	5.85898 ± 0.05049
II	DEM	10.73779 ± 0.1207*	4.27512 ± 0.04004*
III	DEM + 6 Hz (32mA)	12.14546 ± 0.3120*	3.48124 ± 0.04964*
IV	DEM + 6Hz (44mA)	14.57347 ± 0.1909*	3.189515 ± 0.01062*

DEM= Diethyl maleate (844 mg/kg, i.p. - single dose); n =Number of animals in a group.

Values are expressed as Mean ± SD

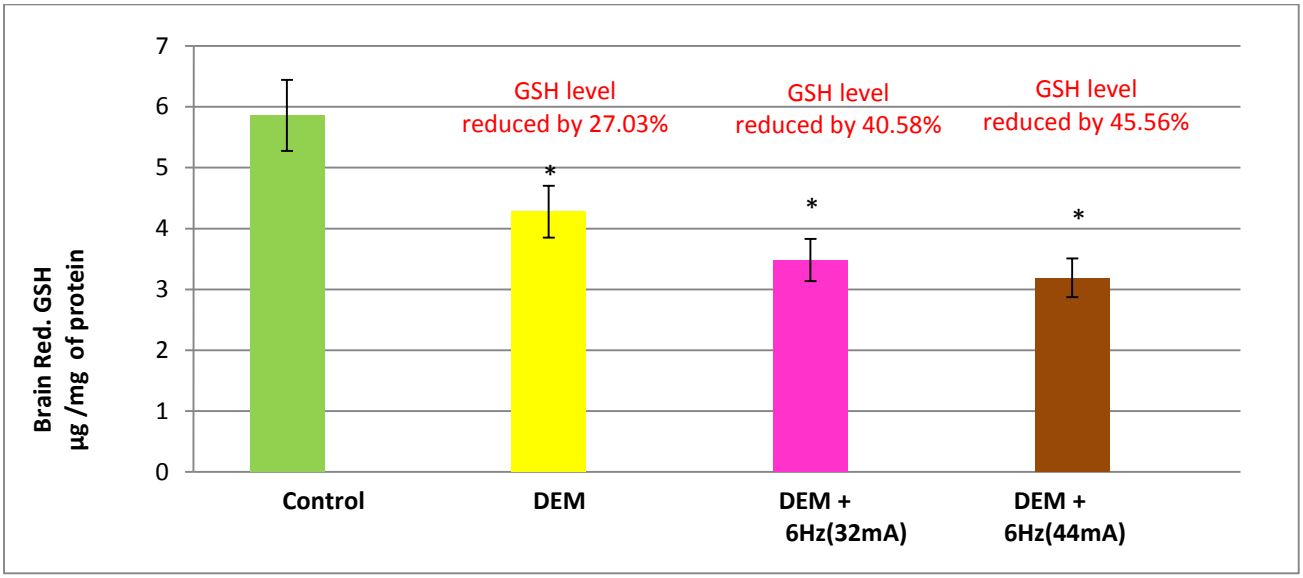
* denotes p< 0.01 when compared with control group (one –way ANOVA followed by Dunnett's t-test). [significant]

Figure 1: Effect of DEM sensitized 6Hz psychomotor seizure on brain malondialdehyde (MDA) level in mice.



*** denotes p< 0.01 when compared with control group (one –way ANOVA followed by Dunnett's t-test). [significant]**

Figure 2: Effect of DEM sensitized 6Hz psychomotor seizure on brain glutathione (GSH) level in mice.



*** denotes p< 0.01 when compared with control group (one –way ANOVA followed by Dunnett's t-test). [significant]**

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