

## Experimental evaluation of anticonvulsant effect of human placental extract in pentylenetetrazole induced convulsions in mice

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### ABSTRACT

**Background:** Human placental extract (HPE) is used in different clinical conditions. Human placental extract (HPE), a folklore medicine, prepared from fresh, healthy human placenta has been in use, as single dose therapy in the treatment of epilepsy in some parts of India. Hence, present study was carried out to explore anticonvulsant property of Human placental extract (HPE) in Pentylenetetrazole (PTZ) induced convulsions in albino mice.

**Methods:** Effects of human placental extract (HPE) (1.0, 1.2 and 1.4 ml / 100 gm body weight) as test drug, sodium valproate (150 mg / kg body weight) as standard and distilled water as control were studied in pentylenetetrazole (PTZ) induced convulsions in albino mouse model. Failure to observe even a single episode of tonic spasm for 5 sec. duration for 1 hour was taken as index of anticonvulsant activity. Onset, duration, complete recovery from convulsion and percent protection was calculated and statistical analysis was carried out using student 't' test.

**Results:** Pretreatment with human placental extract (HPE) administered in the dose of 1.0 ml / 100 gm body weight provided 33.33% and in the doses of 1.2 and 1.4 ml / 100 gm body weight and sodium valproate provided 100% protection from convulsions induced by Pentylenetetrazole in albino mice.

**Conclusion:** Human placental extract (HPE) has shown promising anticonvulsant effect on Pentylenetetrazole induced mouse model.

**Keywords:** Anticonvulsant, Human placental extract (HPE), Sodium valproate, Pentylenetetrazole

### INTRODUCTION

Epilepsy is a disorder of brain function characterized by periodic and unpredictable occurrence of seizures. The term Seizure refers to a transient alteration of behavior due to the disordered, synchronous, and rhythmic firing of populations of brain neurons.<sup>1</sup> Epilepsy is the second most common neurological disorder in India.<sup>2,3</sup> Standard therapy permits control of seizures in approximately 80% of these patients.<sup>4</sup> But even with the best management, optimum seizure control cannot be guaranteed, and the disease remains disabling and stigmatizing.<sup>5</sup>

Although a number of antiepileptic drugs are available, seizures remain uncontrolled in more than 20% of the

patients. Unfavorable responses including drug reactions or poor response to the presently available drugs also indicate a need for development of new drugs or molecules (for treating epileptic seizures) as alternatives.<sup>6</sup> In this context, the anticonvulsant effect of human placental extract (HPE) was taken up for the study.

Traditional folk medicine makes good use of the flora and fauna of a region, including a number of mineral substances. These herbs and animals are used either whole or in part and made up in either simple or complicated preparations and have proved to be effective therapeutic remedies. Some of these medicinal substances of animal as well as human origin have been quite thoroughly studied with modern scientific methods, and

their therapeutic properties have been confirmed although not always explained.<sup>7</sup>

It is known from traditional folk knowledge that the placenta, supporting the baby's growth and development in the mother's womb, contains a wide range of biologically active components. The variety of biological actions of human placental extract (HPE) is a matter of increasing interest. Recent studies revealed that human placental extract (HPE) is a rich source of various bio-active substances like polydeoxyribonucleotides (PDRN), RNA, DNA, peptides, amino acids, enzymes, trace elements, etc.<sup>8</sup>

Human placental extract (HPE) is in use to treat different clinical conditions such as injections for pelvic inflammatory diseases and topical administration in wound healing. Previous studies have shown its antiplatelet, anti-inflammatory, immunomodulating, antioxidant and neuroprotective effect.<sup>7</sup>

In India, besides the use of natural and synthetic medicinal products, some medicines prepared from human parts are employed in curing the diseases. Human placental extract (HPE), a folklore medicine, prepared from fresh, healthy human placenta has been in use as single dose therapy in the treatment of epilepsy in some parts of India. Hence, present study was undertaken to explore anticonvulsant property of Human placental extract (HPE) in Pentylentetrazole (PTZ) induced convulsions in albino mice.

## METHODS

### Animals

Male albino mice weighing between 25 and 40 grams used for the study were obtained from the central animal house, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar, AP. The protocol for the study has been approved by Institutional Animal Ethics Committee (IAEC). The experiment was carried out in post graduate laboratory of Pharmacology department.

### Chemical

#### Pentylentetrazole (PTZ)

Pentylentetrazole (Himedia Laboratories Pvt. Ltd., Mumbai, India) was procured from standard suppliers of chemicals.

### Drugs

#### Sodium valproate

Sodium valproate (Sun Pharma, Mumbai, India) was purchased from pharmacy.

### Human Placental Extract (HPE)

Human placental extract - Placentrex injection manufactured by Albert David limited, India, was purchased from pharmacy. It is an extract of fresh, term, healthy human placenta tissues. Each ml of Placentrex is derived from 0.1 gm of fresh human placenta.

### Method

The study was conducted on male albino mice. The chemical method using pentylentetrazole was employed for comparing the anticonvulsant activity of human placental extract (HPE) with control and standard group of mice. The study was carried out according to the method of H. Gerhard Vogel.<sup>9</sup>

Prior to the experiment, the mice had free access to food and water and were maintained under standard 12 hour light dark cycle. The experiment was carried out at the same time every day (to decrease the variability in epileptic responsiveness from time to time in a day).

Before proceeding with the actual experiment, effective dose of human placental extract (HPE) in pentylentetrazole induced convulsions in mice had been carried out.

### Fixing the effective dose of human placental extract (HPE)

The dose of the test drug human placental extract (HPE) mentioned in literature is up to 45 ml / kg body weight (4.5 gm / kg body weight), given intraperitoneally in mice was found to be safe.<sup>10</sup> As the anticonvulsant dose of human placental extract (HPE) was not known, the study was done to know the effective dose per 100 gm body weight of mouse.

The mice were injected with graded dose increments of human placental extract (HPE). Pairs of mice were administered 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ml / 100 gm body weight of test drug (human placental extract) intraperitoneally. After 30 minutes, pentylentetrazole (PTZ) dissolved in normal saline<sup>11</sup> was administered at the dose of 60 mg / kg body weight subcutaneously and observed for one hour for onset, duration and complete recovery from convulsions and also protection from convulsions, were recorded (Table 1). The doses of 1.2 and 1.4 ml / 100 gm body weight have shown anticonvulsant effect. The doses 1.0, 1.2 and 1.4 ml / 100 gm body weight were chosen for the study.

### Experimental procedure

Thirty male albino mice were divided into 5 groups of 6 each. The mice were marked and kept in separate labeled cages.

**Table 1: Dose response effect of human placental extract (HPE) on pentylenetetrazole induced convulsions on albino mice.**

S. No.	Dose of human placental extract (ml /100 gm body weight)	Convulsions			% Protection
		Onset (sec.) Mean $\pm$ SD	Duration (sec.) Mean $\pm$ SD	Recovery (sec.) Mean $\pm$ SD	
1	0.20	915 $\pm$ 49.49	54.00 $\pm$ 4.23	835 $\pm$ 17.60	0 %
2	0.40	950 $\pm$ 70.71	47.50 $\pm$ 3.87	740 $\pm$ 8.94	0 %
3	0.60	1010 $\pm$ 4.47	41.50 $\pm$ 2.64	685 $\pm$ 8.36	0 %
4	0.80	1086 $\pm$ 18.64	22.00 $\pm$ 2.44	540 $\pm$ 10.95	0 %
5	1.00	1190 $\pm$ 24.89	17.50 $\pm$ 2.23	402 $\pm$ 6.36	0 %
6	1.20	–	–	–	100 %
7	1.40	–	–	–	100 %

n = 2 mice in each group

**Control:** Each mouse was administered 0.5 ml distilled water intraperitoneally.

**Standard:** Each mouse was administered sodium valproate 150 mg / kg body weight intraperitoneally.<sup>(12)</sup>

**Test – 1 (HPE 1.0):** Each mouse was administered human placental extract (HPE) 1.0 ml / 100 gm body weight intraperitoneally.

**Test – 2 (HPE 1.2):** Each mouse was administered human placental extract (HPE) 1.2 ml / 100 gm body weight intraperitoneally.

**Test – 3 (HPE 1.4):** Each mouse was administered human placental extract (HPE) 1.4 ml / 100 gm body weight intraperitoneally.

Half an hour after administration of distilled water to control group, sodium valproate to standard group and human placental extract to test groups of mice, pentylenetetrazole (PTZ) 60 mg / kg body weight was administered subcutaneously over the scruff of the neck and observed for one hour.

The mice were placed in separate cages and were observed for 1 hour. Failure to observe even a single episode of tonic spasm for 5 sec. duration for 1 hour was taken as index of anticonvulsant activity. The time taken for the onset, duration and complete recovery from convulsions induced by pentylenetetrazole (PTZ), was recorded. Simultaneously, the number of episodes of convulsions was also recorded.

The results were expressed as Mean  $\pm$  SD. The statistical tool, student 't' test, was used to analyze the data.

## RESULTS

The effect of drugs on mice was observed for one hour after administration of pentylenetetrazole and the readings were recorded.

The mice were observed for onset, duration and recovery from convulsions and percentage protection from convulsions were also recorded and analyzed.

It was observed that all the mice in control group developed convulsions. Human placental extract (HPE) at a dose of 1.0 ml / 100 gm body weight (Test – 1 group) provided 33.33% protection while at doses 1.2 (Test – 2 group) and 1.4 ml / 100 gm body weight (Test – 3 group) provided 100% protection from pentylenetetrazole induced convulsions in mice (Table 2).

The mean time of onset of convulsions was increased and time of duration and recovery from convulsion were decreased in 1.0 ml / 100 gm body weight of human placental extract (HPE) (Test – 1 group) when compared to control group (Table – 2) and it was found statistically highly significant ( $P < 0.001$ ).

The Standard, Test – 2 and Test – 3 groups of mice did not develop any convulsions.

It was also noted that in Control group one mouse died due to prolonged second episode of convulsion, whereas, no death occurred in other groups.

**Table 2: Effect of human placental extract (HPE) on pentylenetetrazole induced convulsions in albino mice.**

S. No.	Group	Convulsions			
		Onset (sec.) Mean $\pm$ SD	Duration (sec.) Mean $\pm$ SD	Recovery (sec.) Mean $\pm$ SD	% Protection
1	<b>Control</b> Distilled water 0.5 ml / mouse	882.58 $\pm$ 21.59	58.50 $\pm$ 5.26	860.50 $\pm$ 17.20	0 %
2	<b>Standard</b> Sodium valproate 150 mg / kg body weight	–	–	–	100 %
3	<b>Test – 1 (HPE 1.0)</b> Human Placental Extract 1.0 ml / 100 gm body weight	1260.60 $\pm$ 16.04*	12.33 $\pm$ 4.30*	360.33 $\pm$ 8.38*	33.33 %
4	<b>Test – 2 (HPE 1.2)</b> Human Placental Extract 1.2 ml / 100 gm body weight	–	–	–	100 %
5	<b>Test – 3 (HPE 1.4)</b> Human Placental Extract 1.4 ml / 100 gm body weight	–	–	–	100 %

n = 6 mice in each group

\* P &lt; 0.001 Highly significant, when Test group compared with control group.

## DISCUSSION

Pentylenetetrazole is a chemoconvulsant, which induces seizures by the inhibition of GABA<sub>A</sub> receptors and widely accepted experimental model for absence seizure.<sup>13,14</sup>

Administration of sodium valproate completely inhibited pentylenetetrazole induced convulsions and mortality of animals. As an anticonvulsant sodium valproate act by multiple mechanisms, blockade of voltage-dependent sodium channels, augmentation of gamma-aminobutyric acid (GABA)-mediated inhibition. Sodium valproate has been shown to be an effective agent in ameliorating the symptoms of generalized absence seizures via blockade of voltage dependent t-type calcium channels. Thus, our results are in consonance with previous reports and sodium valproate served as a standard control in the present study.

The experiment was carried out in two stages in the laboratory. Initially, the dose response was studied with human placental extract (HPE) to fix the effective anticonvulsant dose and later anticonvulsant effect of three doses (1.0, 1.2 & 1.4 ml / 100 gm body weight) of

human placental extract (HPE) was compared with that of control group and standard group.

The percent protection of Test – 2 (HPE 1.2) and Test – 3 (HPE 1.4) groups was 100% and with Test – 1 (HPE 1.0) group was 33.3% when compared to Control group in pentylenetetrazole induced convulsions in mice. There were no subsequent episodes of seizures and none died in all Test groups and Standard group compared to Control group. There was increase in the time of onset and decrease in the time of duration and recovery from convulsions in Test – 1 (HPE 1.0) group compared to control group.

Statistical analysis of Test – 1 (HPE 1.0) group as compared to Control group was highly significant P < 0.001.

The results suggest that human placental extract (HPE) has anticonvulsant property.

The mechanism by which pentylenetetrazole produce convulsions, has not been completely understood. Generally accepted mechanism by which pentylenetetrazole exerts its action is by acting as an

antagonist at the picrotoxin sensitive site of the GABA<sub>A</sub> receptor complex.<sup>15</sup> Papp et al. found that pentylenetetrazole also increases calcium influx and sodium influx, both of which depolarize the neuron.<sup>16</sup>

Another mechanism suggested by other studies has demonstrated that oxidative stress might be involved in the pathophysiology of pentylenetetrazole (PTZ) induced epilepsy.<sup>17-21</sup> Pentylenetetrazole (PTZ) at a convulsant dose decreases antioxidant enzymes activity and glutathione levels and increases lipid peroxidation in brain.

In the present study human placental extract (HPE) has shown significant protection from pentylenetetrazole (PTZ) induced convulsions in mice. The probable anticonvulsant mechanism of action of HPE may be due to its increased antioxidant enzymes (superoxide dismutase and catalase) activity and glutathione levels and decreased lipid peroxidation in brain.<sup>22</sup>

The various other mechanism of action of human placental extract (HPE) as an anticonvulsant may be either due to agonistic action on GABA<sub>A</sub> receptor complex or due to decrease in calcium influx and sodium influxes in neuron, as the mechanism of convulsant action of pentylenetetrazole is related to it. It requires further study to establish the hypothesis.

## CONCLUSION

The present study showed that human placental extract (HPE) possesses anticonvulsant activity against pentylenetetrazole induced convulsions in albino mice. Further studies are required to unearth full potential of human placental extract (HPE) as an anticonvulsant.

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