

## THE PHARMA INNOVATION - JOURNAL

### Protective Effect of *Benincasa hispida* against Pentylentetrazol-induced Kindling in Mice

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This study aimed to identify the protective effect of MEBH using the chemo-convulsive, Pentylentetrazol kindling model in mice. The methanol extract of *Benincasa hispida* (MEBH) was studied at dose levels of 100, 300 & 1000mg/kg. The extract at 300mg/kg reduced the percentage of incidence of convulsions from the mid phase of the kindling protocol. MEBH significantly increased the latency to the onset of seizures and prolonged the onset of death at a dose level of 300mg/Kg. From the present study, we can conclude that the methanol extract of *Benincasa hispida* shows protection upon chronic administration depicting anticonvulsant activity.

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**Keyword:** *Benincasa hispida*, Pentylentetrazol kindling, Anticonvulsant Potential.

#### 1. Introduction:

In India the fruit of *Benincasa hispida*, belonging to Cucurbitaceae family is an important ingredient for the Ayurvedic medicine “Kusmanda lehyam” which is used as a rejuvenative tonic and also said to improve intellect and physical strength. The fruit juice is used in folklore medicine & also recommended by Ayurvedic texts for nervous disorders (insanity, epilepsy), GIT problems (dyspepsia, burning sensation, peptic ulcer, piles & constipation, hunger), respiratory diseases (cough, asthma), heart disease, cataract, syphilis, sexual dysfunction, verimifuge, diabetes, urinary disease (difficulty in urination & bladder stones), excessive thirst and antidote for alcohol and mercury poisoning<sup>[1,2]</sup>. The anticonvulsant activity previously carried out with the methanolic extract of *Benincasa hispida* was acute and the results reveal certain degree of

potential anticonvulsant activity in PTZ, MES and strychnine models<sup>[3]</sup>. Kindling is a model of epilepsy produced by repeated administration of an initially subconvulsive electrical or chemical stimulus that results in an increase in seizure activity, culminating in a generalized seizure. The electrographic and behavioral components of kindled seizures are thought to be similar to human partial-onset seizures, as the focal component of the kindled seizure can progress into a generalized seizure. In this model, the effect of drugs on both focal and generalized seizure types can be quantified. Kindling can be produced via electrical or chemical stimulation of many, but not all brain sites<sup>[4,5]</sup>. The PTZ kindling is generally used as a laboratory model of human partial complex epilepsy<sup>[5-7]</sup> whereas single administration of a submaximal dose of PTZ is thought to reflect absence and myoclonic seizures in humans<sup>[8]</sup>. Hence the present study

was designed to identify the protective effect of MEBH in PTZ-kindling seizure model.

## 2. Materials and Methods

### 2.1 Preparation of fruit Extract

The fruit of *Benincasa hispida* was purchased from local market during the month of July, 2006. The cuticle of the fruit was peeled. The fruit was cut and freed from seeds and endocarp. The pulp of the fruit was cut into small pieces and mashed well using an electric juicer. The ground pulp was macerated using 1:4 dilution of methanol and water, respectively for 7 days. Then the mother liquor was separated from the marc by using a muslin cloth and allowed to evaporate using a rotovac drier. The semisolid brownish colored extract thus obtained was stored in a cool, dry place. The yield of the extract was 10.2% w/w.

### 2.2 Animals

Male Swiss albino mice (18-25g) were used for the present study. The animals were inbred and obtained from the animal house facility of the JSS College of Pharmacy, Ootacamund. The animals were housed under a strict 12/12h light/dark cycle with lights on at 8:00am. The animals were maintained with standard pellet food (Amrut feeds, Mumbai) and drinking water available *ad libitum* throughout the experiment. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC).

### 2.3 Preparation of Test Substance and Drugs

The methanol extract of *Benincasa hispida* (hereafter termed as MEBH), diazepam and pentylenetetrazol (PTZ) was dissolved in 0.9% saline. All the drugs were prepared freshly before the start of the experiments.

### 2.4 Dose Administration and Dose Volume

MEBH, chemoconvulsants, diazepam and the vehicle (normal saline) were administered intraperitoneally with a disposable syringe of 1-5 ml capacity fitted with a disposable needle of 26 G. The dose volume for mice was 0.1 ml/10g.

### 2.5 PTZ-induced Kindling in Mice

Male Swiss albino mice were used to produce kindling. The animals were given a total of 11 treatments (**Fig.1**) with 50 mg/kg PTZ on every second day (Monday, Wednesday, and Friday). Mice were observed for 30 min after PTZ administration for seizure intensity which was evaluated using the following scale <sup>[9]</sup>;

- 0 - no response
- 1 - ear and facial twitching
- 2 - myoclonic body jerks
- 3 - clonic forelimb convulsions
- 4 - generalized clonic convulsions  
(turn onto a side position)
- 5 - generalized clonic tonic convulsions

The animals were considered to be fully kindled after reaching stage 4 or 5 seizures on three consecutive PTZ treatments during the experimental period. The animals which did not exhibit stage 4 or 5 seizures during the 21 day kindling period were excluded from the study.

### 2.6 PTZ-challenge test in kindled mice

The animals (i.e. the fully kindled mice) were left without any treatment for four days (day 22-25). On day 26, the mice were administered with their respective treatment and 30 min later challenged with 75 mg/kg of PTZ <sup>10</sup>. The time of onset of myoclonic seizures (s), duration of clonic seizures (s), onset of tonic seizures (s) and death (s) were recorded for 30 min.

### 2.7 Statistical Analysis

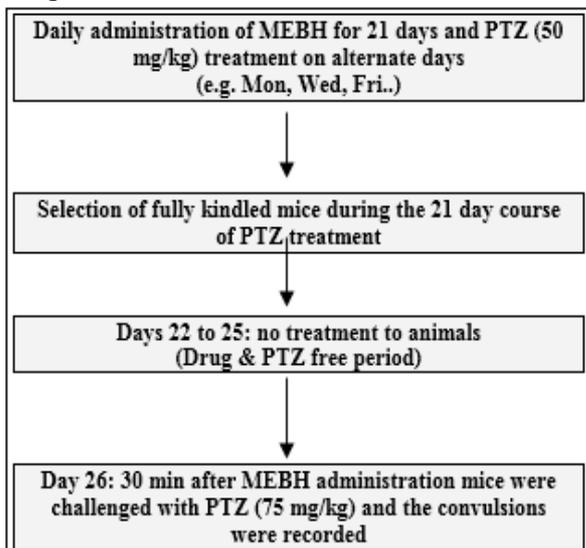
All values were expressed as mean  $\pm$  sem. Statistical analysis was carried out using One-Way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using Graph Pad prism v.4 software. P values less than 0.5 were considered as statistically significant.

## 3. Results:

### 3.1 Protective Effect of MEBH on Percentage of Incidence of Convulsion in PTZ-Induced Kindling in Mice:

The effect of pretreatment with MEBH on the percentage of incidence of convulsions in PTZ kindling model is shown in **Table 1**. Diazepam

show a consistent significant ( $p < 0.05$ ) reduction in the % of convulsion while MEBH 300 too showed but only from the mid phase of the experiment but not comparable to that of diazepam.



**Fig 1:** Protocol for PTZ kindling and challenge studies in mice.

### 3.2 PTZ-Challenge Test:

After a treatment free period of 3 days, the fully kindled mice were challenged with PTZ. MEBH 300 mg/kg only showed significant ( $p < 0.01$ ) increased latency to onset of tonic seizures,  $1257 \pm 234.4s$  when compared to control,  $412.5 \pm 36.9s$  (Table 2). The onset of death time too was significantly prolonged. In the case of diazepam, the positive control used did not inhibit any signs of convulsion (myoclonic, clonic or tonic) and none of the mice died too at the end of the observational period. Out of 6 mice only 2 mice survived upon MEBH 300 mg/kg treatment while there was 100 % mortality in MEBH 100 and 1000 mg/kg treated mice when challenged with PTZ.

**Table 1:** Effect of MEBH on % of incidence of convulsion upon chronic administration of PTZ on alternate days.

Day	Control (PTZ kindled)	Diazepam (5 g/kg)	MEBH (mg/kg)		
			100	300	1000
1	63 ± 8.03	10 ± 4.47**	63 ± 8.03	63 ± 9.55	60 ± 7.30
3	63 ± 6.15	13 ± 4.22**	83 ± 8.03	70 ± 4.47	57 ± 6.15
5	70 ± 4.47	13 ± 4.22**	90 ± 4.47	77 ± 8.03	67 ± 9.89
7	73 ± 6.67	10 ± 4.47**	97 ± 3.33	67 ± 12.29	53 ± 8.43
9	73 ± 4.22	7 ± 4.22**	93 ± 4.22**	67 ± 4.22	63 ± 3.33
11	87 ± 6.67	7 ± 4.22**	93 ± 4.22	73 ± 6.67	50 ± 6.83**
13	87 ± 4.22	0 ± 0.00**	83 ± 8.03	63 ± 3.33**	63 ± 3.33**
15	87 ± 4.22	0 ± 0.00**	87 ± 4.22	63 ± 3.33**	77 ± 3.33
17	83 ± 6.15	0 ± 0.00**	90 ± 6.83	63 ± 3.33*	83 ± 3.33
19	77 ± 6.15	0 ± 0.00**	80 ± 7.30	60 ± 5.16	77 ± 3.33
21	93 ± 4.22	0 ± 0.00**	70 ± 4.47**	60 ± 5.16**	60 ± 0.00**

Values are mean ± SEM; N=number of mice per group; \* $p < 0.05$ , \*\* $p < 0.01$  vs. control. One Way ANOVA followed by Dunnett’s multiple comparison test.

#### 4. Discussion:

Animal models of seizures induced by certain chemoconvulsants have been used widely to study the mechanism of investigational drug [11]. From the studies carried out previously [3], it is clear that MEBH produces anticonvulsant activity only in selective models probably through various mechanisms. It was of interest to subject MEBH in a specific model of epilepsy resembling or reflecting the epilepsy in humans. The model chosen was PTZ kindled model in mice. Kindling shares many behavioral features with complex partial seizures in humans [5-7]. The kindling model, nevertheless, permits the discrimination of transient effects arising simply in response to seizure from longer-lasting plastic effects that are thought to underlie a permanent increase in seizure disposition (typically measured 24 h and 1 month after stimulation, respectively) [12]. In the experiment conducted to evaluate the effect of MEBH in attenuating the seizures or to postpone the full blown kindling, the results were disappointing. Although MEBH 300 mg/kg showed potential to reduce the percentage of convulsion, the results were not comparable to that of diazepam, wherein the percentage was around 0-20% throughout the experimental period. Similarly challenging the mice with PTZ after a treatment free period of 3 days after day 21, only MEBH 300 mg/kg showed significant protection against onset of tonic and death time. But at the same hand diazepam was able to produce 100 % protection. Thus from the above kindling model it may be suggested that MEBH may not have significant antiepileptic activity although in acute models certain degree of anti-seizure or anticonvulsive activity was observed.

#### 5. Conclusions:

The present study undertook an ambitious aim to evaluate the antiepileptic activity of the methanol extract of *Benincasa hispida* based on the previous investigations and also on the traditional claim of this fruit in Indian system of medicine. The results reveal that MEBH possesses good anticonvulsant activity at a dose level of 300mg/kg. The conduct of further

pharmacological studies is necessary to exploit the mechanism of action through which the constituents of the extract exert anticonvulsant action.

**Table 2:** Effect of MEBH on the convulsive parameters on PTZ kindled mice when challenged with PTZ (90 mg/kg; i.p.) on day 26 after a drug free period of 3 days.

Treatment	Dose (mg/kg; p.o.)	Onset of seizures (s)			Death time (s)
		Myoclonic	Clonic	Tonic	
Control	-	74.0 ± 4.97	60.7 ± 4.86	412.5 ± 36.9	426.8 ± 35.3
Diazepam	5	-	-	-	1800 ± 0.0**
MEBH	100	103.5 ± 3.74**	48.7 ± 2.2	355.5 ± 17.0	363.7 ± 17.4
	300	97.3 ± 6.87*	66.8 ± 6.69	1257 ± 243.4*	1303.0 ± 226.3*
	1000	89.0 ± 4.77	56.3 ± 4.21	545.5 ± 59.48	591.5 ± 79.3

Values are mean ± SEM; N=number of mice per group; \*p<0.05, \*\*p<0.01 vs. control. One Way ANOVA followed by Dunnett's multiple comparison test.

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