



## Neuroprotective and Nootropic Activity of *Carica Papaya* Seeds on Diabetes induced Cognitive Decline in Rats

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### Abstract

The aim of present study is to investigate neuroprotective and nootropic activity of Petroleum Ether Extract of *Carica papaya* seeds (PEECPS) on diabetic induced cognitive decline rats. Rectangular maze and morris water maze models were used to evaluate nootropic activity and neuroprotective effects were studied by estimating acetyl cholinesterase (AChE), malondialdehyde (MDA), superoxide dismutase (SOD), nitric oxide (NO), catalase (CAT) and glutathione (GSH) levels in the brains of diabetic rats. In rectangular maze and morris water maze models, 400 mg/kg of PECPS were shown the significant effect compared with diabetic control on day 75. Significant decrease in AChE ( $P<0.001$ ), MDA ( $P<0.01$ ), NO ( $P<0.05$ ) and significantly ( $P<0.01$ ) increased levels of SOD, CAT and GSH with PECPS (200 and 400 mg/kg) compared with diabetic control. There is a need of further studies on *Carica papaya* seeds as it showed protection against diabetes induced cognitive decline to reveal its mode of action.

**Key words:** *Carica papaya* seeds, morris water maze, neuroprotective, nootropic, radial arm maze, cognitive, diabetes

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### 1. Introduction

Diabetes mellitus (DM) is a worldwide health problem afflicting millions in both developed and developing countries. DM is a chronic metabolic disorder characterized by hyperglycemia resulting from either low level or resistance to insulin. DM causes chronic kidney failure, blindness, high blood pressure, and premature coronary artery diseases. The complications of DM such as cognitive

dysfunction, Alzheimer's, and dementia are less addressed or recognized [1].

Cognitive impairment due to diabetes mainly occurs in two periods, during the first 5-7 years of life when the brain system is in development and the period when the brain undergoes neurodegenerative changes due to aging [2]. In population based studies, patients with type 2DM have an increased risk of cognitive impairment, dementia and neurodegeneration. Stress is one of the primary reason of referral to psychiatrists and is a cause of numerous brain disorders. Oxidative stress and reactive oxygen species (ROS) are major contributors and sources of the aging process and linked to neurodegenerative disorders such as Alzheimer's and Parkinson's disease [3]. The brain is particularly exposed to oxidative stress because of its high metabolic rate and low antioxidant defense [4]. The accumulation of free radicals progressively damages the brain's morphology and results in memory impairments. Oxidative stress becomes evident by increased lipid peroxide, nitric oxide (NO) levels as well as decreased glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) levels [5]. Neurotransmitter functions which are altered in DM include decreased acetylcholine (ACh) production, serotonin turnover, dopamine activity and increased norepinephrine [2]. Substantial experimental evidence exists for a relation between the decline in cholinergic functions and dementia [6].

DM associated cognitive decline is a new term anticipated to facilitate research in diabetic encephalopathy. Hyperglycemia, oxidative stress and [6]. Anti-diabetic and psychotropic drugs are associated with several adverse effects and this has encouraged the need for exploring drugs without side effects especially, from traditional system of medicine.

The scientific evidences related to *Carica papaya* as anti-diabetic, anti-hyperlipidemic, hypoglycemic, nephroprotective, anti-nociceptive, anti-inflammatory, anti-ulcer, antioxidant activity, acetyl cholinesterase inhibiting activity, depression, and anticonvulsant activity [7]. Since the plant was reported to be used by the traditional system of medicine as anti-diabetic, acetyl cholinesterase inhibiting activity and believed to promote memory and intelligence, authors have premeditated this study to explore the nootropic and neuroprotective potential of petroleum ether extract of *C. papaya* seeds (PECPS) *Linn* on DM induced cognitive decline animal model.

## 2. Materials and Methods

### 2.1. Chemicals

Scopolamine (Cadila Health Care Ltd), Donepezil (Alkem Laboratories Ltd), Glibenclimide (Sigma laboratories), USA), Diagnostic Kits (Bio Lab, India), MDA, CAT and Streptozotocin (Sigma Aldrich, USA), were purchased from local market. Other chemicals used were of analytical grade.

## 2.2. Plant Materials and Preparation of Extracts

The fruits of *Carica papaya* were collected from local market, Warangal, Telangana State, India, and authenticated by Dr. V. S. Raju, Department of Botany, Kakatiya University, Warangal. Dried seeds were grinded in a domestic mixer grinder and coarse powder was separated by sieving. The sample was extracted with Petroleum Ether and dried at 40°C. The obtained powder was chocolate in color with aromatic odor [8].

## 2.3. Animals

Wistar albino rats (150-200gms) were used for this study and procured from mahaveer enterprises, Hyderabad. The animals were housed into group of six animals per cage, maintained at 24±1 °C with relative humidity 45-55 % and 12:12 hour's dark and light cycle. The animals had free access to food (standard chow pellets) and water *ad libitum*. All the experimental procedures were approved by IAEC (IAEC NO: 1047/ac/07/CPCSEA), Vaagdevi College of Pharmacy, Warangal,

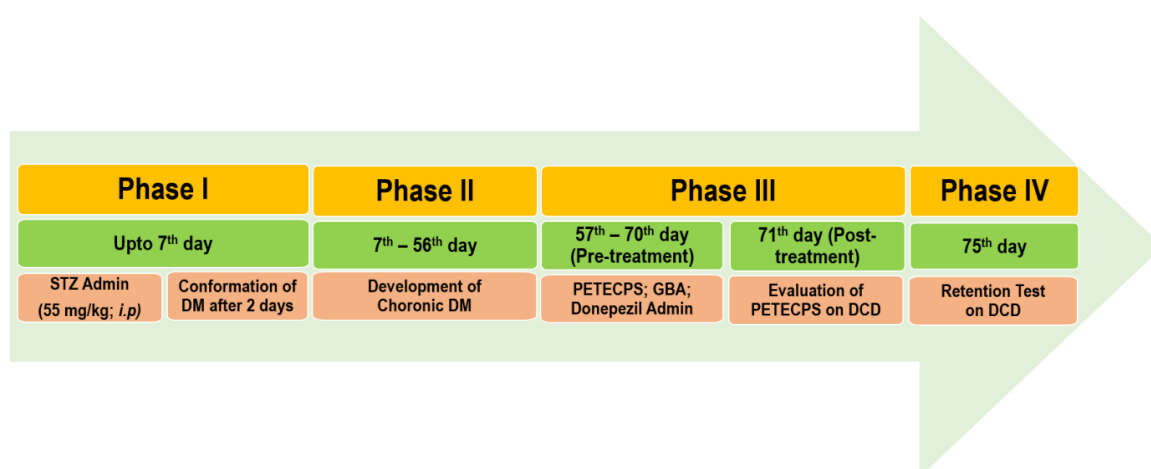
T.S., India. Experimental study design is illustrated in figure 1.

## 2.4. Induction of DM

Streptozotocin (STZ) dissolved in cold citrate buffer (pH 4.45) was administered intraperitoneally (55 mg/kg) to induce diabetes. After 48 h of STZ injection, the blood samples were collected from retro-orbital plexus of rats under light, using fine glass capillary in Eppendorf tubes and serum glucose level was estimated by glucose oxidase and peroxidase enzymatic method. Animals with serum glucose more than 250 mg/dl considered as diabetic and used for further study [9].

## 2.5. Nootropic Study

The PECPS was tested for nootropic studies and the selected animals were divided in to six groups with six animals in each. The details of groups were used to nootropic studies.



**Figure 1.** Experimental study design.

## 2.6. Rectangular Maze Test

The maze consists of completely enclosed rectangular box with an entry and reward chamber appended at opposite ends. The box is partitioned with wooden slots into blind passages leaving just twisting corridor leading from the entry to the reward chamber. The animals were trained prior to the experiment by familiarizing with the rectangular maze for a period of 10 min for 2 hand chosen for the experiment. Four transfer latency readings were recorded and the average was considered as learning score for that animal. Lower scores of assessment indicate efficient learning while higher scores indicate poor learning in animals. The time taken by the animals to reach the reward chamber from the entry chamber was noted on day 71(Training trial) and 75 (Retention trial) [10].

## 2.7. Morris Water Maze Test

On 71<sup>st</sup> day, the animals were tested in a spatial version of Morris water maze. The apparatus consists of a circular pool (90 cm in diameter and 50 cm in height) of water with a featureless inner surface. Day one of the experiment was dedicated to swimming training for 60 secs in the absence of the platform. During four consecutive days, the rats were given the trial session with the platform in place. If the rat locates the platform, it was allowed remaining on it for 10 secs. If it does not, it was placed for 10 secs and then removed from the platform. The day after final training trial sessions (on day 5), the rats were individually subjected to a probe trial session in which the platform was removed

from the pool and were allowed to swim for 120 secs to search for it. On 71<sup>st</sup> day, the animals were tested in a spatial version of Morris water maze and the latency time was determined [11].

## 2.8. Neurochemical Study

On 76<sup>th</sup> day, the animals were sacrificed, and the brains were isolated and weighed. Whole brain was rinsed with ice cold saline and homogenized by making 20 mg of the tissue per ml in chilled phosphate buffer (pH 7.4). The homogenates were centrifuged at 800 rpm for 5 min at 4 °C to separate the nuclear debris. Again, the supernatant was centrifuged at 5000 rpm for 20 min at 4 °C. The obtained supernatant was then used for neurochemical study. The protein concentration was estimated by Lowry method using bovine serum albumin as the standard [12].

## 2.9. Estimation of Cholinergic Status in the Rat Brain

The cholinergic marker, AchE was estimated in the whole brain according to the method of Ellman. A total of 0.4 ml supernatant was added to a cuvette containing 2.6 ml phosphate buffer (pH 8) and 100 µL of DTNB (5, 5'-dithiobis-2-nitrobenzoic acid). The contents of the cuvette were mixed thoroughly and absorbance was measured at 412 nm by a spectrophotometer. Basal value reading was recorded when the absorbance value becomes stable. The substrate of 20 µL of acetyl thiocholine iodide was added and the changes in absorbance were recorded for a

period of 10 min at intervals of 2 min. Change in the absorbance per min was thus determined. The mean change in absorbance was considered for calculation using following formula and AchE activity was measured as  $\mu\text{M/L/min/gm}$  of tissue [13].

$$R = 5.74 \times 10^{-4} \times \frac{\Delta A}{C_o}$$

Where, R = Rate, in moles substrate hydrolyzed per min per gm of tissue;  $\Delta A$  = Change in absorbance per minutes;  $C_o$  = Original concentration of tissue (20 mg/ml)

#### 2.10. Hydrogen Peroxide Scavenging Assays (Catalase Activity)

The assay mixture consists of 3 mL of hydrogen peroxide, 1 mL of phosphate buffer and 0.05 mL of the supernatant of the tissue homogenate. The change in absorbance was recorded for 2 min in the intervals of 30 sec at 240 nm using UV spectrophotometer. The results were expressed as micromoles of  $\text{H}_2\text{O}_2$  decomposed per minute per mg protein [14].

Scavenged percentage hydrogen peroxide =

$$(A_0 - A_1 / A_0) \times 100$$

Where,  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance of the samples [15].

#### 2.11. Lipid Peroxide levels

Thiobarbituric acid reactive substance (TBARS) levels were measured as an index of malondialdehyde (MDA) production, which is an end product of lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) to form a red colored complex. The 0.5 ml of Tris hydrochloric acid was added in 0.5 ml of supernatant and incubated at 37 °C for 2 h.

After incubation, 1 ml of 10 % trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min, and then 1 ml of 0.67 % TBA was added to 1 ml of above mixture and heated for 10 min on boiling water. After cooling, 1 ml of double distilled water was added and absorbance was measured at 532 nm. The MDA concentrations of the samples were derived from the standard curve prepared using known amounts of MDA and expressed as nmol MDA/mg of protein [16].

#### 2.12. Total Nitric Oxide Levels

To 100  $\mu\text{l}$  of supernatant, 500  $\mu\text{l}$  of Greiss reagent was added and absorbance was measured at 546 nm. Nitrite concentration was calculated using a standard curve for sodium nitrite and expressed as ng/mg of protein [17].

#### 2.13. Superoxide Dismutase (SOD) Levels

To 100  $\mu\text{l}$  of supernatant, 1 ml of sodium carbonate (1.06 % in water), 0.4 ml of nitro blue tetrazolin (24 mmol/L; NBT) and 0.2 ml of EDTA (0.037 % water) was added and 0 min reading was taken at 560 nm. Reaction was initiated by addition of 0.4 ml of hydroxylamine hydrochloride (1 mM), incubated at 25 °C for 5 min and the reduction of NBT was measured at 560 nm. SOD level was calculated using the standard calibration curve, and expressed in  $\mu\text{g/mg}$  of protein [18].

#### 2.14. Glutathione levels (GSH)

1 ml of supernatant was precipitated with 1 ml of sulphosalicylic acid (4 %). The samples were kept at 4 °C for about 1 h and centrifuged at 1200 rpm for 15 min at 4 °C. The assay

mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (pH 7.4) and 0.2 ml DTNB, Ellman's reagent, in a total volume of 3 ml. The yellow color developed was read immediately at 412 nm and expressed as  $\mu\text{g}/\text{mg}$  of protein. The GSH concentrations of the samples were derived from the standard curve prepared using known amounts of GSH and expressed as  $\text{ng}/\text{mg}$  protein [16].

### 3. Results and Discussion

In diabetic controls, the percentage of spontaneous alteration was significantly ( $P<0.05$ ) decreased in retention trial compared with normal controls (NC). The PECPS-I, glibenclimide, PECPS-II and donepezil treated diabetic animals showed significant increase in percentage spontaneous alterations in retention trial compared with diabetic controls (DC) [Table 1]. During training, no significant difference of transfer latency (TL) was

observed. In retention trial of DC, the TL was found to be significantly ( $P<0.01$ ) increased compared with NC. The TL in retention trial were observed significantly ( $P<0.05$ ,  $P<0.01$ ,  $P<0.001$ ) decreased with PECPS-I & II and donepezil treated animals compared with DC whereas not significant with glibenclimide treated animals [Table 1]. In diabetic patients, the incidence of dementia appears to be doubled in elderly subjects [19]. A significant increase in cognitive function was observed in type 2 diabetic rats treated with the extract in the present study. There were no earlier reports on the effect of PECPS on cognitive function. Many mechanisms explains how diabetes could increase risk of dementia including hyperglycemia, insulin resistance, oxidative stress, advanced glycation products, inflammatory cytokines, microvascular, and macrovascular complications [20]. The earlier reports of PECPS include antidiabetic,

**Table 1.** Effect of PETECPS on %SA in Y maze arm and TL in morris water maze of diabetes induced cognitive decline rats.

Groups	Dose (mg/kg)	% SA (Rectangular maze)		TL (Morris water maze)	
		71 <sup>st</sup> day	75 <sup>th</sup> day	71 <sup>st</sup> day	75 <sup>th</sup> day
Group -I	---	30.34 $\pm$ 2.32	31.19 $\pm$ 2.09	50.99 $\pm$ 2.59	45.12 $\pm$ 2.27
Group -II	120	45.22 $\pm$ 2.29	38.32 $\pm$ 2.46 <sup>a</sup> *	75.80 $\pm$ 4.37	52.54 $\pm$ 4.88 <sup>a**</sup>
Group -III	10	35.84 $\pm$ 4.86	45.20 $\pm$ 5.42 <sup>b</sup> *	61.28 $\pm$ 2.41	50.84 $\pm$ 3.32
Group -IV	5	40.54 $\pm$ 4.34	52.98 $\pm$ 3.96 <sup>b</sup> ***	46.54 $\pm$ 3.82	43.60 $\pm$ 2.20 <sup>b***</sup>
Group -V	200	39.62 $\pm$ 3.42	45.51 $\pm$ 1.81 <sup>b</sup> *	60.42 $\pm$ 2.51	46.54 $\pm$ 4.02 <sup>b*</sup>
Group -VI	400	44.01 $\pm$ 3.82	47.23 $\pm$ 3.95 <sup>b</sup> **	59.41 $\pm$ 3.82	44.67 $\pm$ 1.54 <sup>b**</sup>

n=6, values are mean  $\pm$  SEM, \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ ; <sup>a</sup>Compared to normal control group, <sup>b</sup>Compared to diabetic control group

antioxidant and anti-inflammatory properties, responsible for this study. Venkateshwarlu *et al.*, reported that extract of *C. papaya* significantly decreased the level of blood glucose in STZ induced diabetic animals [21]. In diabetic controls, AchE levels were significantly increased ( $P<0.001$ ) compared with NC, suggesting cholinergic dysfunction. Diabetic animals treated with PECPS-I & II and donepezil significantly decreased the levels of AchE, whereas glibenclimide group showed insignificant effect [Table 2]. In DC,

TBARS was significantly ( $P<0.001$ ) increased compared with NC. Reduction in TBARS were observed treatment with PECPS-I ( $P<0.05$ ) & II ( $P<0.001$ ) donepezil ( $P<0.001$ ) and glibenclimide ( $P<0.01$ ) [Table 3]. In diabetic group, a significant decrease in SOD, CAT and GSH levels was observed, compared with NC. The PECPS-II treated animals showed significant ( $P<0.01$ ) improvement in SOD, CAT, and GSH levels, with PECPS-I significant increase in CAT ( $P<0.05$ ) and GSH ( $P<0.01$ ) levels with no significant increase in

**Table 2.** Effects of PETECPS on AchE activity of diabetes induced cognitive decline in rats.

Groups	Dose(mg/kg)	AchE Levels ( $\mu$ M/min/mg of protein)
Group -I	---	4.36 $\pm$ 0.26
Group -II	120	6.12 $\pm$ 0.47 <sup>a***</sup>
Group -III	10	6.45 $\pm$ 0.42
Group -IV	5	4.03 $\pm$ 0.45 <sup>b***</sup>
Group -V	200	5.15 $\pm$ 0.25 <sup>b*</sup>
Group -VI	400	5.00 $\pm$ 0.67 <sup>b**</sup>

n=6, values are mean  $\pm$  SEM, \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ ; <sup>a</sup>Compared to normal control group, <sup>b</sup>Compared to diabetic control group

**Table 3.** Effect of PETECPS on Total nitrites, MDA, CAT, SOD and GSH levels in diabetes induced cognitive decline in rats.

Groups	Dose (mg/kg)	Total nitrites (ng/mg of protein)	MDA Levels (M/mg of tissue)	CAT ( $\mu$ g/mg of protein)	SOD ( $\mu$ g/mg of protein)	GSH (ng/mg of protein)
Group -I	---	115.20 $\pm$ 0.32	23.00 $\pm$ 0.57	5.34 $\pm$ 0.30	106.10 $\pm$ 0.34	13.42 $\pm$ 0.69
Group -II	120	237.31 $\pm$ 1.05 <sup>a***</sup>	45.24 $\pm$ 2.16 <sup>a***</sup>	2.42 $\pm$ 0.32 <sup>a**</sup>	45.80 $\pm$ 2.50 <sup>a**</sup>	3.10 $\pm$ 0.01 <sup>a**</sup>
Group -III	10	160.30 $\pm$ 0.38 <sup>b***</sup>	42.00 $\pm$ 2.36 <sup>b*</sup>	3.25 $\pm$ 0.20 <sup>b**</sup>	82.55 $\pm$ 1.08 <sup>b**</sup>	10.53 $\pm$ 0.52 <sup>b**</sup>
Group -IV	5	215.00 $\pm$ 0.98 <sup>b***</sup>	40.12 $\pm$ 0.27 <sup>b***</sup>	2.46 $\pm$ 0.28 <sup>b*</sup>	60.33 $\pm$ 0.48 <sup>b*</sup>	5.25 $\pm$ 0.45 <sup>b**</sup>
Group -V	200	203.10 $\pm$ 0.96 <sup>b***</sup>	42.00 $\pm$ 2.98 <sup>b*</sup>	3.13 $\pm$ 0.32 <sup>b*</sup>	50.31 $\pm$ 5.23 <sup>b*</sup>	7.16 $\pm$ 0.09 <sup>b**</sup>
Group -VI	400	150.50 $\pm$ 1.17 <sup>b***</sup>	41.00 $\pm$ 2.09 <sup>b**</sup>	3.23 $\pm$ 0.47 <sup>b**</sup>	84.41 $\pm$ 1.02 <sup>b**</sup>	11.93 $\pm$ 1.32 <sup>b**</sup>

n=6, values are mean  $\pm$  SEM, \* $P<0.05$ , \*\* $P<0.01$ s, \*\*\* $P<0.001$ ; <sup>a</sup>Compared to normal control group, <sup>b</sup>Compared to diabetic control group



diabetic animals. Glibenclimide showed significant ( $P<0.01$ ) increase in SOD and CAT levels but no significant effect on GSH levels. Donepezil showed significant increase only in SOD ( $P<0.01$ ) and GSH ( $P<0.01$ ) levels [Table 3]. Treatment with PECPS-I & II and glibenclimide and donepezil showed significant ( $P<0.001$ ) reduction on brain NO level in diabetic animals [Table 3]. The *C. papaya* seed extracts were studied for its cognitive behavior, anxiety, depression, stresses, and convulsions induced by pentylenetetrazole and maximum electroshock method. To explain these effects, the effect of PECPS were also studied mediated by MAO inhibitory activity, which there was an increase in the concentration of dopamine, norepinephrine (NE), Ach, and serotonin. Literature report reveals that papaya contains good amount of tyrosine, phenylalanine (precursor of NE) as well as tryptophan [22]. Hence, it was worth investigating the effects of PECPS on diabetes induced cognitive decline in experimental animals along with its role in oxidative stress and AchE activity. The results of the present study showed significant improvement in spatial reference memory and TL, suggesting nootropic activity of PECPS in diabetes induced cognitive decline models. Oxidative stress in brain generates free radicals like superoxide anion, hydroxyl radical, and hydrogen peroxide, which act on polyunsaturated fatty acids in brain, thereby propagating the lipid peroxidation. The major antioxidant and oxidative free radical scavenging enzyme like GSH, SOD, and catalase plays an important role in reducing

oxidative stress in brain [23]. Oxidative damage to various brain regions constitutes the long-term complications, morphological abnormalities, and memory impairments. In the present study, TBARS levels were significantly increased whereas GSH, SOD, and CAT levels were markedly reduced in the brains of diabetic control. PECPS might have protective effect against diabetes induced cognitive decline due to reduced oxidative stress. The antioxidant properties of PECPS might help to ameliorate the cognitive dysfunction in diabetic animals. The enzyme AChE degrades the neurotransmitter acetylcholine. The AChE were significantly risen in diabetic rats [16]. Treatment with PECPS attenuated increase in AchE, ameliorated cognitive decline, cholinergic dysfunction, reduced oxidative stress, and NO in the brain of diabetic animals, which may find clinical application in treating neuronal deficit in the diabetic patients. From the above results it was concluded that diabetes induced cognitive decline was improved by the treatment with *C. papaya* seeds in rats and further investigation is needed to know the exact mechanism of cognition enhancement for developing lead compounds and to overcome the limitations of the current work.

#### 4. Conclusion

In conclusion, our results demonstrated that extract of *C. papaya* shows nootropic and neuroprotective activity against STZ induced cognitive decline in animal model. However, further studies by using pure compounds of



this plant are needed to clarify if the plant has neuroprotective effects or not.

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