

NEUROPROTECTIVE EFFECTS OF MOMORDICA CHARANTIA ON SCOPOLAMINE INDUCED ALZHEIMER'S DISEASE

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ABSTRACT

Momordica charantia, commonly known as bitter guard or bitter melon, is an important medical plant of India. Its fruits have potent anti-oxidant activity due to the presence of tannins, vitamin C and flavonoids. The aim of this study was to investigate the beneficial effect of the ethanolic extract of fruits of *momordica charantia* on memory impairment in Swiss albino mice. scopolamine (1.4mg/kg,i.p) was administered to induce amnesia and Donepezil (5mg/kg, oral) used as standard drug. Rectangular maze test, Morris water maze test,

locomotor activity and pole climbing test were conducted to evaluate learning and memory parameters. Various biochemical parameters such as acetyl cholinesterase (AChE), TBARs assay, catalase activity, and DPPH assay were also assessed. The present study demonstrates that ethanolic extract of *momordica charantia* had potential therapeutic effect on improving the anti-amnesic activity in mice through inhibiting lipid peroxidation and decreasing acetyl cholinesterase activity in brain.

KEYWORDS: *momordica charantia*, scopolamine, TBAR, AChE,**INTRODUCTION**

Alzheimer's disease (AD) is a neurodegenerative disease causing memory loss and dementia, which mostly affects the elderly population (Francis PT *et al.*, 1999).

The pathophysiology of AD is complex including defective beta-amyloid (A β) protein metabolism, abnormalities of glutaminergic, adrenergic, serotonergic, dopaminergic neurotransmission and the potential involvement of inflammatory and oxidative pathways (Kang SY *et al.*, 2005).

The impairment of memory in scopolamine induced animal model is associated with altered status of brain oxidative stress. Strong evidence supporting the involvement of oxidative stress within the forebrain cholinergic system has been suggested (Wilson JX *et al.*, 1997). The drugs with anti-oxidant effects might be beneficial for preserving brain function. Anti-oxidant enzymes are involved in the reduction of oxidative stress (Masaki HS *et al.*, 1995).

Anti-oxidant enzymes display the reduced activities in the affected brain region of Alzheimer's disease patients (Vandana). Moreover, the reduction in the level of intracellular oxidized protein under these conditions has been associated with the improvement of cognitive and/ or psychomotor functions. Thus, the efforts have been directed to find therapeutic agents that could reduce the oxidative damage and promote a functional recovery in degenerative disorders.^[5]

Momordicacharantia grows in tropical and sub tropical regions of the world belonging to the family cucurbitaceae. This is commonly known as bitter guard or bitter melon. It is an important dietary source of vitamin C and also provide vitamin A (Sook young lee *et al.*, 2009).

It also contain inorganic mineral elements such as potassium, calcium, and zinc. It mainly contains secondary metabolites such as Alkaloids, Tannins, Flavonoids, Saponins, Reducing compounds, Glycosides, Phylobatannins, steroids and terpenoids. (Bakare R.I *et al.*, 2010).

Momordicacharantia tested for various pharmacological activities like anthelminthic, Anti viral, Anti microbial, Anti fertility activity, Hypoglycemic activity, Insecticide activity, DNA synthesis inhibitor, Cytotoxic activity, Anti cancer activity, Anti bacterial activity, and Anti tumor activity (Potawale S, *et al.*, 2008).

The aqueous extract of *momordica charantia* is a potent inhibitor of lipid peroxide formation and scavenging of hydroxyl and superoxide radical in vitro (Alireza Rezaeizadeh *et al.*, 2011).

MATERIALS AND METHODS

Animals. Swiss mice of male sex weighing 20–25 g were used in the present study. They were acclimatized to laboratory conditions for 2 days before behavioral studies. The Institution Animals Ethics Committee (IAEC) had approved the experimental protocol, and

care of animals was taken as per guide lines of CPCSEA, Department of Animal Welfare, and Government of India (Kulkarni P. D *et al.*, 2011).

TABLE

Group-I	control	Vehicle(0.1% CMC)
Group-II	Disease control	Scopolamine(1.4 mg/kg) I p.
Group-III	Standard	Donepezil(5mg/kg) oral+Scopolamine(1.4 mg/kg) i p
Group-IV	Dose 1	Ethanollic extract of momordicacharantia(200mg/kg) oral + Scopolamine(1.4 mg/kg) I p.
Group-V	Dose 2	Ethanollicextract of momordicacharantia(400mg/kg) oral + Scopolamine(1.4 mg/kg) I p.

Drugs

Scopolamine (Cadila Healthcare pvt. Ltd), and Donepezil (Alkemlaboratories Ltd.) were purchased. Scopolamine and selegiline were diluted with distilled water.

Momordicacharantia extract was prepared.

Preparation of the extract

The fruits of *Momordica* were chopped into small pieces and dried under shade at roomtemperature for seven days. The dried fruits were powdered and passed through the sieve (coarse10/40). The dried powder was extracted with 95%v/v ethanol for 10 days using maceration. Then filtered through watsman filter paper and dry the extraction. Then store it.

Behavioural Tests

All the animals were trained for 4 days before drugs administration. **Rectangular Maze Test:** Assessment of learning and memory can be effectively done by this method. The maze consists of completely enclosed rectangular box with an entry and reward chamber appended at opposite ends. The box is partitioned with wooden slats into blind passages leaving just twisting corridor leading from the entry to the reward chamber(Indumathy S. *et al.*,2010). Animals were trained prior to the experiment by familiarizing with the rectangular maze for a period of 10 min for 2 h. Well-trained animals were taken for the experiment. Transfer latency (time taken to reach the reward chamber) was recorded. For each animal, four readings were taken and the average is taken as learning score (transfer latency) 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 1, 3, 5, 7, and 9 (Goverdhan p *et al.*, 2012).

Morris Water Maze Test

Morris water maze was used to assess learning and memory in experimental mice. There are several advantages of Morris water maze over other models of learning and memory including absence of motivational stimuli such as food and water deprivation, electrical stimulations, and buzzer sounds (Vogel H. G *et al.*, 2002, Morris R *et al.*, 1984). Briefly, it consists of a circular water tank, filled with opaque water, and one centimeter submerged platform. First, animals were trained to locate the platform. During acquisition, trial escape latency time (ELT), time measure to locate the hidden platform, was noted as an index of acquisition. Each animal was subjected to the four acquisition trials per day for 4 consecutive days. The time spent by the animal, searching for the missing platform in target quadrant Q2 with respect to other quadrant (Q1, Q3, and Q4) on 5th day, was noted as an index of retrieval. For studying the effect of drug on acquisition, the drug solution was administered before acquisition trial (Goverdhan p *et al.*, 2012, Saraf M. K *et al.*, 2011).

Locomotor Activity

The locomotor activity of drug can be studied using actophotometer which operates on photoelectric cells which are connected in circuit with a counter when the beam of light falling on photocell is cut off by the animal, then a count is recorded. Animals are placed individually in the activity cage for 10min and the activity was monitored. The test is done before 30 min and after the drug administration. The photocell count is noted and decrease or increase in locomotor activity is calculated (Goverdhan p *et al.*, 2012, Vogel H. G *et al.*, 2002).

Pole Climbing Test

When an electrical stimulus is given to animal, it tries to escape from it and move to the near safe place. This equipment is designed in such a way to climb the pole when stimulus is generated. Prior to the experiment, animals were trained. Training and testing is conducted in a 25 × 25 × 40 cm chamber that is enclosed in a dimly light, sound attenuated box. Scrambled shock is delivered to the grid floor of the chamber. A smooth stainless steel pole, 2.5 cm in diameter, is suspended by a counter balance weight through a hole in the upper centre of the chamber. A micro switch is activated when the pole is pulled down by 3 mm. With weight greater than 200 gm. A response is recorded when a mice jumps on the pole and activates micro switch. The activation of light and speaker together is used as conditioned stimulus. Each animal was placed six times per day (Vogel H. G *et al.*, 2002).

Dissection and Homogenization

On day 9, after behavioral assessments, animals were sacrificed by cervical dislocation. The brains were removed. Each brain was separately put on ice and rinsed with ice-cold isotonic saline. A (10% w/v) homogenate was prepared in 0.1M phosphate buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 15 minutes and aliquots of supernatant were separated and used for biochemical estimation (Yu Z. F *et al.*, 1997).

Biochemical Tests

AchE Estimation

The cholinergic marker, acetylcholinesterase, was estimated in the whole brain according to the method of Ellman method. Ellman's reagent is 5,5-dithiobis(2-nitrobenzoate) and it is also abbreviated as DTNB.

This homogenate was incubated for 5 min with 2.7mL of phosphate buffer and 0.1mL of DTNB. Then, 0.1mL of freshly prepared acetylthiocholine iodide (pH 8) was added and the absorbance was read at 412nm (Abhinav K *et al.*, 2010, Kumar A *et al.*, 2009).

Thiobarbituric Acid Reactive Substances (TBARS) Assay

This assay is used to determine the lipid peroxidation. Aliquots of 0.5mL distilled water were added with 1 mL of 10% trichloroacetic acid and were added with 0.5mL of brain tissue homogenate. This is centrifuged at 3000 rpm for 10 min. To the 0.2mL supernatant, 0.1mL thiobarbituric acid (0.375%) was added. Total solution is placed in water bath at 80°C for 40 min and cooled at room temperature. Absorbance was read at 532nm (Kaur I. P *et al.*, 2006).

Catalase Activity

Catalase activity was assessed by the method of Luck (Luck H *et al.*, 1971), wherein the breakdown of hydrogenperoxide is measured. In this 3mL of H₂O₂ phosphate buffer was added to 0.05mL of the supernatant of the tissue homogenate. The absorbance was recorded at 240nm using Perkin Elmer Lambda 20 spectrophotometer. The results were expressed as micromoles of H₂O₂ decomposed per minute per mg protein (Kumar A *et al.*, 2009).

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay:

In this, measurement is made from the bleaching of purple-coloured methanol solution of DPPH. To the 1000 µL of diverse conc. of the sample, 4mL of 0.004% methanolic solution of

DPPH was added. After 30 min incubation, absorbance was read at 517 nm. Inhibition of free radical by DPPH in % was calculated in the following way:

$$\% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100,$$

Blank: absorbance of control reaction.

Sample: absorbance of test sample. (Kaur I. P *et al.*, 2006).

RESULTS

Behavioural Tests

Rectangular Maze Test: The activity of *Momordica charantia* was evaluated using rectangular maze. The mice in all treatment groups except scopolamine-treated group showed lower transfer latency on 5th, 7th day and 9th day compared to 3rd day of the same group as well as with the scopolamine group which was given in Figure 1.

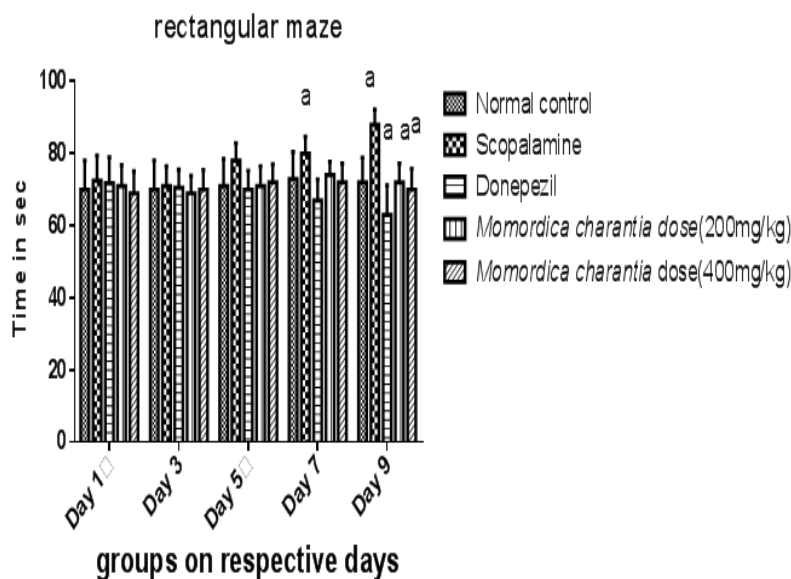


Figure 1: Rectangular maze test. Effect of Ethonolic extract of *Momordica charantia* on latency time compared to the disease control group. (Mean±SD, n = 6). Graph showing mean± SD of latency time in seconds. aP < 0.01 compared with corresponding values of disease control.

Morris WaterMaze Test

The activity of *Momordica charantia* was evaluated using Morris water maze. The mice treatment groups except scopolamine-treated group showed significant transfer latency on 4th day with platform and on 5th day without platform which was given in Figure 2.

This indicates memory enhancing capacity of the *Momordica charantia*. Donepezil (5 mg/kg) treated for successive 8 days acts as positive control, possessed significant ($P < 0.05$) decrease in transfer latency when compared to disease control (scopolamine) using dunnet's test.

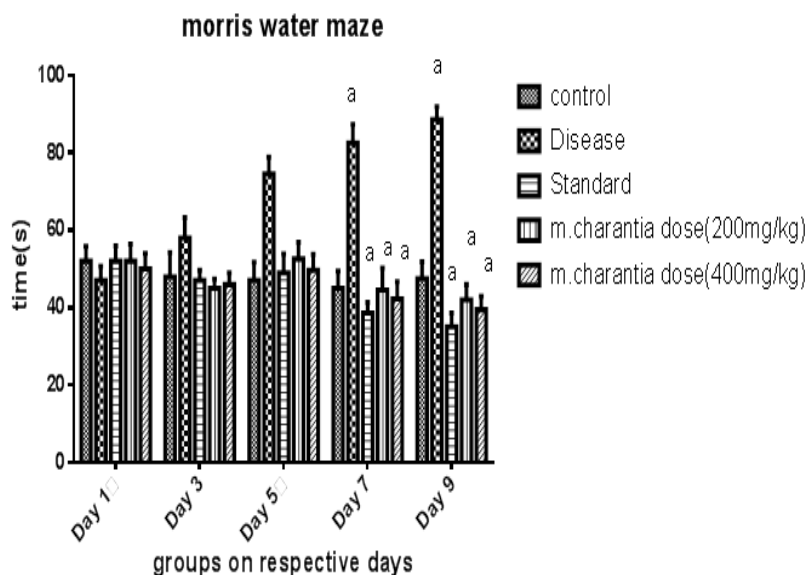


Figure: 2 Morris watermaze test. Effect of Ethonolic extract of *Momordica charantia* on latency time compared to the disease control group. (Mean \pm SD, $n = 6$). Graph showing mean \pm SD of latency time in seconds. $aP < 0.01$ compared with corresponding values of disease control.

Locomotor Activity

The activity of Ethonolic extract of *Momordica charantia* was evaluated using photoactometer. The mice showed significant transfer latency on 5th, 7th day compared to the 9th day in all treatment groups except scopolamine treated group which was given in Figure 3. This Donepezil (5mg/kg) treated successive 8 days acts as positive control, possessed significant ($P < 0.05$) decrease in number of crossings which is comparable to the other treatment groups

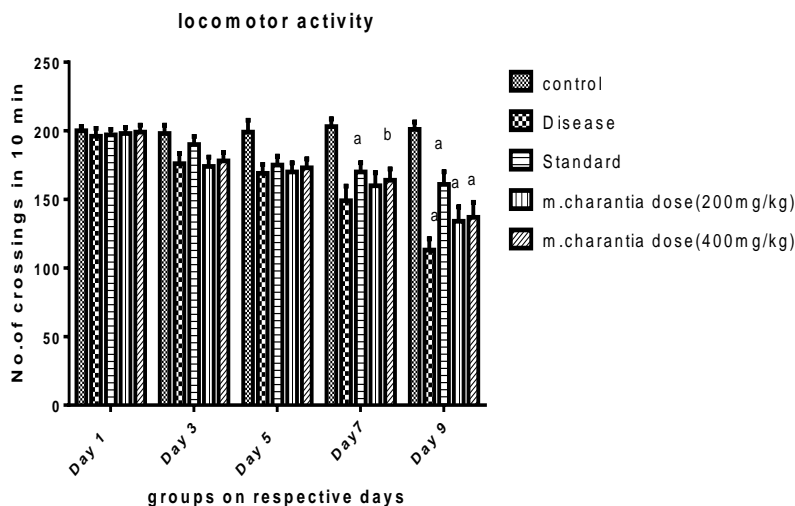


Figure 3: Locomotor activity. Effect of Ethonolic extract of *Momordica charantia* on latency time compared to the disease control group. (Mean \pm SD, $n = 6$). Graph showing mean \pm SD of latency time in seconds. a) $P < 0.01$, b) $P < 0.05$ compared with corresponding values of disease control.

Pole Climbing Test. The values show that there was a significant difference that has been observed on days 5, 7 and 9 compared to the 1 and 3. Scopolamine-treated group took more time whereas the control and drug-treated groups showed less time to reach the pole in pole climbing apparatus.

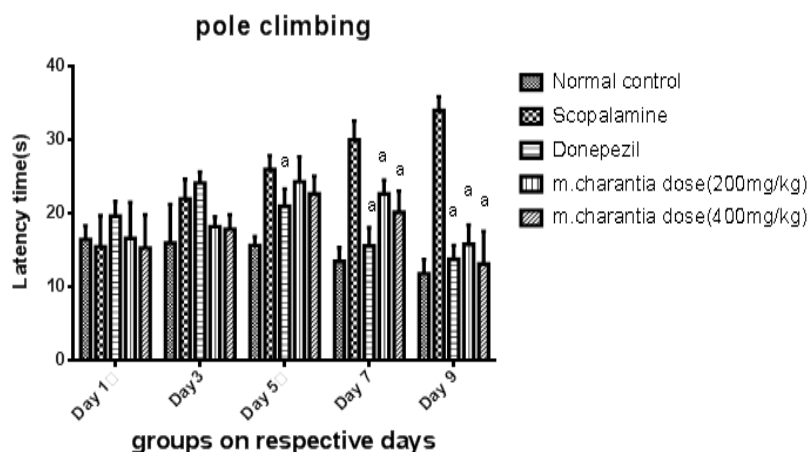


Figure 4: Pole climbing test: Effect of on Ethonolic extract of *Momordica charantia* latency time levels compared to the disease control group (Mean \pm SD, $n = 6$). Graph showing mean \pm SD of latency time in seconds. a) $P < 0.01$ compared with corresponding values of disease control.

Biochemical Tests

AchE Estimation. Scopolamine treatment significantly increased the brain AchE level compared to control group (Figure 5). Standard drug (donepezil) and test drugs (Momordica

charantia extract) treatment significantly inhibited the brain AchE level compared to their corresponding scopolamine treated groups.

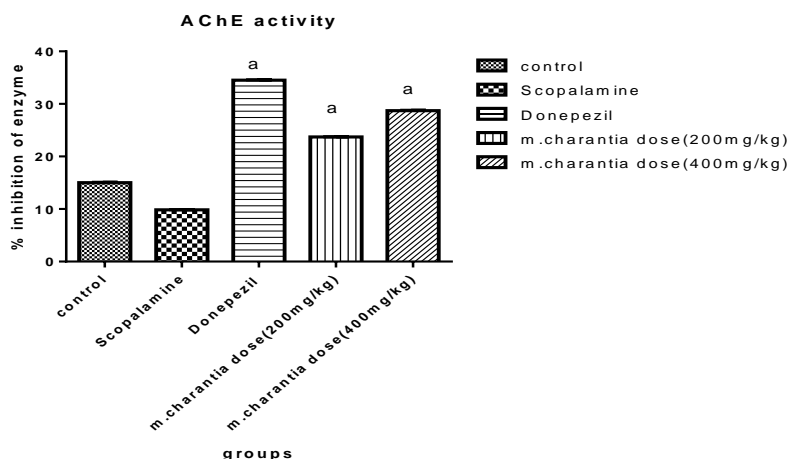


Figure 5: AchE estimation. Effect of Ethanolic extract of *Momordica charantia* on AchE levels compared to the disease control group. (Mean± SD, n = 6). Graph showing mean± SD of %inhibition of AchE enzyme. aP < 0.01 compared with corresponding values of disease control.

TBARS Assay. Scopolamine treatment significantly increased the brain MDA level compared to control group (Figure 6). Standard drug (donepezil) and test drugs (Momordica charantia extract) treatment significantly (P < 0.05) decreased brain MDA level compared to their corresponding scopolamine treated groups.

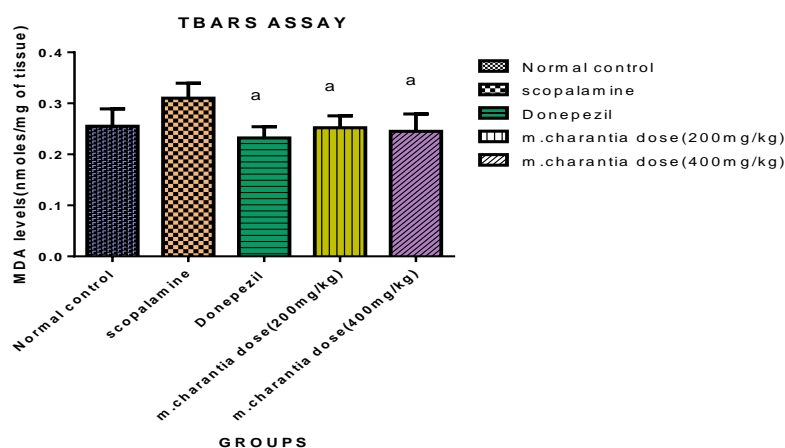


Figure: 6 TBARS assay. Effect of Ethonolic extract of *Momordica charantia* on malondialdehyde levels compared to the disease control group. (Mean± SD, n = 6). Graph showing mean± SD of malondialdehyde levels. aP < 0.01 compared with corresponding values of disease control.

Catalase Activity

Catalase levels were decreased in scopolamine- treated groups compared to the normal control group (Figure 7). Significant ($P < 0.05$) difference has been found in drug-treated groups. Synergistic effect was observed which is comparable with the standard group than individual drug-treated groups.

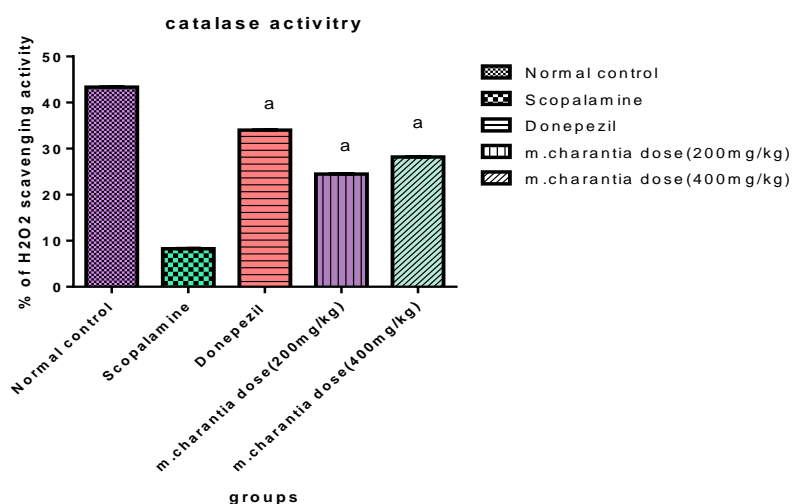


Figure 7: Catalase activity. Effect of Ethonolic extract of *Momordica charantia* on catalase activity compared to the disease control group. (Mean±SD, $n = 6$). Graph showing mean± SD of %H₂O₂ scavenging activity. $aP < 0.01$ compared with corresponding values of disease control.

DPPH Assay. Antioxidant levels were decreased in scopolamine-treated group compared to the control group (Figure 8). Drug-treated groups showed significant ($P < 0.05$) difference compared to the disease control group.

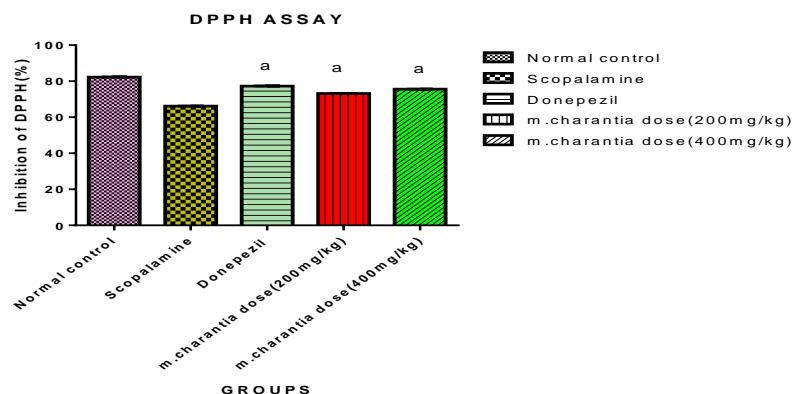
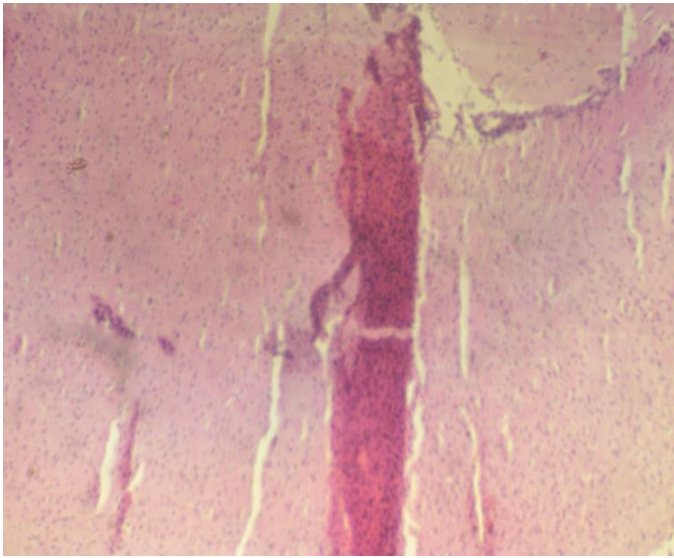
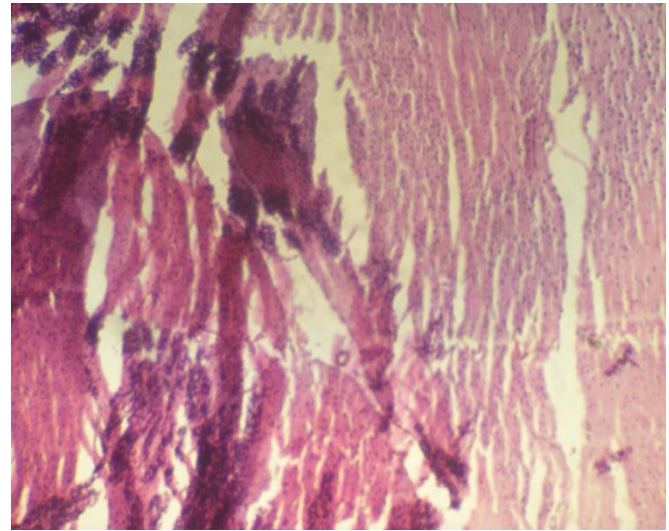


Figure 8: DPPH assay. Effect of Ethonolic extract of *Momordica charantia* on inhibition of DPPH compared to the disease control group (Mea± SD, $n = 6$). Graph showing

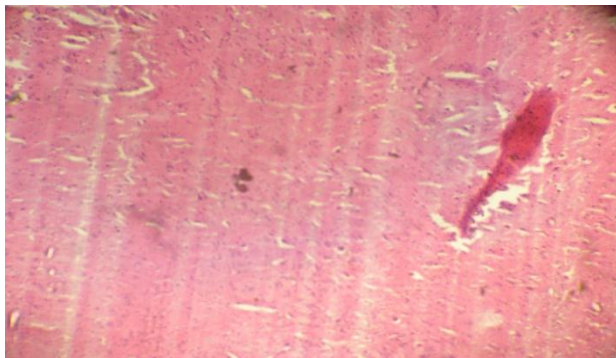
mean \pm SD of %inhibition of DPPH. $P < 0.01$ compared with corresponding values of disease control.



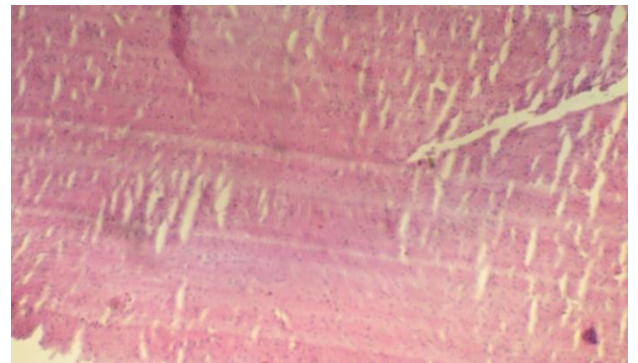
(a)



(b)



(c)



(d)



(e)

Histopathological studies. These Figures (a), (b), (c), (d), and (e) are normal control, scopolamine (disease control), donepezil (standard), *Momordica charantia* extract (200mg/kg), *Momordica charantia* extract (400mg/kg) respectively, representing the histological sections of the brain tissue showing neurological lesions.

Histopathological Studies. From Figure it is clearly visible that in disease control group the degenerated cells are more compared to other groups. This will be indicated by the gaps in slides. The extract-treated groups are in between the normal control and disease control groups.

DISCUSSION

Alzheimer's disease (AD) is a neurodegenerative disease causing memory loss and dementia, which mostly affects the elderly population. It is characterized by aphasia, apraxia and agnosia with the loss of memory as the main symptom. Despite the severity and prevalence of the disease, allopathic system of medicine is yet to provide a satisfactory drug, Therefore, we were motivated to explore the potential of medicinal plants to manage this deadly disease.

In the present study ethanolic extract of *Momordica charantia* administered orally for 9 days improved learning and memory of mice significantly in both the exteroceptive behavioral models employed.

Oxygen free radicals are implicated in the process of age related decline in cognitive performance might be responsible for the development of Alzheimer's disease in elderly persons. *Momordica charantia* has been reported to possess antioxidant property(). The neuroprotective effect of *Momordica charantia* may be attributed to its antioxidant property by the virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function.

Immunohistochemical studies suggested existence of chronic inflammation in certain regions of the brain in AD patients. Since inflammation can be damaging to host tissue, it was hypothesized that anti-inflammatory drugs might be inhibiting both the onset and the progression of Alzheimer's disease. This hypothesis is supported by the observation that indomethacin(NSAID) halted the progressive memory loss seen in AD patients. Moreover, it has also been observed that elderly patients suffering from AD showed reductions in symptoms of AD upon chronic use of anti-inflammatory drugs. Anti-inflammatory action of

Momordica charantia might also be contributing to the observed memory enhancing activity of *Momordica charantia*.

Recently, several reports have shown a strong link between high cholesterol levels and high incidence of Alzheimer's disease. Clinical studies suggested that the net brain cholesterol concentration is regulated by serum cholesterol level and that there is a crosslink between the central nervous system and peripheral cholesterol pools(). It has been reported that chronic oral administration of ethanolic extract of *Momordica charantia* significantly decrease lipid levels, such as cholesterol and triacylglyceride(TAG), in serum and liver in rats.

From the behavioral test, that is, rectangular maze test and morris water maze test, it is clearly seen that there was a general decrease in the transfer latency in in all treated groups compared to the scopolamine treated group. The memory loss effect of scopolamine is more prominent compared to the control group. In comparison with Donepezil, the extract treated groups had most equal performance against memory loss. Meanwhile locomotor activity and poleclimbing avoidance tests are done which also indicate the learning ability.

The major antioxidant and oxidative free radical scavenging enzymes like glutathione, SOD, and catalase play an important role in reduce oxidative stress in brain. In this study, from the DPPH assay antioxidant levels are estimated. These enzymes levels are decreased in the scopolamine treated group compared to the control group. The enzyme levels are almost equal in extract treated group and the standard group.

In the present study mice after scopolamine treatment showed a significant increase in the brain levels of malondialdehyde, which is the measure of lipid peroxidation and free radical generation. In the drug treated groups, there is a significant decrease in the levels of melondialdehyde which is equal to the standard group. In all tests maximal effect was observed at the dose of 400mg/kg.

Thus, a combination of antioxidant, anti-inflammatory and cholesterol lowering activities exhibited by *Momordica charantia* may all be eventually responsible for the memory improving effect observed in the present study.

CONCLUSION

In conclusion, the present study demonstrated that ethanolic extract of *Momordica charantia* had potential therapeutic effect on improving the anti-amnesic activity in mice through

inhibiting lipid peroxidation, augmenting endogenous antioxidant enzymes, and decreasing acetylcholinesterase(AChE) activity in brain.

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