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# DETERMINATION OF POSSIBLE EFFECT OF (WATERMELON) CITRULLUS LANATUS SEED EXTRACT AGAINST SCOPOLAMINE INDUCED AMNESIA IN RATS

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## ABSTRACT

The present study designed to investigate the effect of Citrullus Lanatus (Watermelon) seed extract on pharmacological properties in Scopolamine-induced Amnesia in rats. A total of 20 female spragwe dawley rats were investigated into 5 equal groups as: group 1 (Normal control group) treated with distilled water (2ml/kg i.p.), Group 2 treated with Scopolamine received Scopolamine 1mg/kg for 15 days, Group 3 (Scopolamine+Citrullus Lanatus seed extract treatment group) received 1mg/kg bodyweight/day for 15 days, Group 4 (Scopolamine+Citrullus Lanatus seed extract) received 1ml/kg+100mg/kg bodyweight/day for 15 days, group 5 (Intact group or Per SE group) received Citrullus Lanatus seed extract of 300mg/kg bodyweight/day for 15 days. The Citrullus Lanatus (Watermelon) seed extract significantly restored learning impairments and memory functions in Scopolamine-induced in the Morris water maze test, elevated plus maze, Grip strength, and biochemicals tests in rats. Citrullus Lanatus seed has repairing effects on memory and behavioural disorders produced by Scopolamine and may have beneficial effects in the treatment of Amnesia disease.

**KEYWORDS:** Acetyl Cholinesterase, Amnesia, Glutathione, Scopolamine, Citrullus Lanatus (Watermelon) seed extract.

## INTRODUCTION

Amnesia (Amnesic syndrome), is deficit in memory caused by injury in brain and also due to use of several drugs. Due to disease that affects the brain and also effects the functions of memory. As the limbic system controls the emotions and memories, any injury in brain that

form the limbic system such as hypothalamus and hippocampus may cause amnesia. Different types of amnesia are reported in amnesia patient. Amnesia usually caused by brain trauma caused due to the blow on head is referred as **Anterograde amnesia,** in which patient facing problem to remembering information. However, the patient involves inability to remember the events that experienced in past. Amnesia is a temporary loss of memory occurs. In traumatic amnesia there is a loss of memory due to accidental blow on the head. In case of segregation that is rare and patients usually forget their past with identity. Other types of amnesia termed as infantile amnesia in which the patient not able to recall early childhood events. In the condition of post-hypnotic amnesia, one cannot remember the events during the hypnosis whereas in the case of **source amnesia.** In the condition of when the person cannot remember faces known as **Prosopagnosia** and this is either acquired or the person is born with Prosopagnosia.

It is characterized by memory impairment and relentless development of cognitive decline. Pathological examination of Alzheimer's disease brain reveals extensive atrophy, collection of neuro-fibrilator complication and deposition of β- Amyloid (Aβ Plaques). Due to the age changes into the brain may reductions in cerebral blood flow, synapse density also decreases in grey as well as white matter. Combination factors such as infection, reduced blood circulation and genetic factors maybe it can cause the Alzheimer's disease.

The cholinergic neuronal system which has been plays an important role in learning as well as memory in humans also in animals. Use of many nootropic agents such as Piracetam like oxiracetam, aniracetam, and metrifonate are enhanced in memory disorders. However, due to the adverse effects associated with such drugs their uses are limited. Nootropics agents including Piracetam, Aniracetam, and Cholinesterase inhibitors such as Donepezil are presently used for the enhancement of the cognitive skills as well as for improving memory, mood and behaviour of the patient.

Scopolamine induced amnesia model in rats is most preferred for evaluating nootropic agents and this also has been used this model in screening of anti-amnestic drugs. Basically, the Scopolamine are nonselective muscarinic cholinergic antagonist which is centrally acting on cholinergic agent that causing memory impairment in rats also in humans AChE inhibitors such as Physostigmine, Tacrine and Donepezil which have a antagonize effects of scopolamine on spatial memory which is established on the basis of the certain behavioural parameters.

Plants such as *Bacopa monniera*, *Withania somnifera*, *Tinospora cordifolia* and *Acorus calamus* are systematically searched for their effect onto the cognitive function of the brain. These are grouped under the general class of Medhaya Rasayana (chemical substances) which have to counter the neurodegenerative changes in associated with aging which is beneficial in promoting the brain power. Physostigmine a prototype of AchE inhibitor has been used to improve memory and cognitive function for patients with Alzheimer disease. Physostigmine significantly improved measure of memory and cognition in placebo-controlled studies. The clinical value of physostigmine is low due to very short half-life approximately 30 minutes. Now a days reversible AchE inhibitor such as Tacrine HCl (Cognex) and Donepezil HCl (Aricept) which are approved by the FDA for treatment of mild and moderate dementia which are associated with Alzhiemer disease. The use of Tacrine is limited due to hepatotoxicity issues and high-cost factor.

(Watermelon) is a large, oval, round or oblong shape that belongs to the family Cucurbitacea. Watermelon have a very rich supply of vitamins and parts or areas of the world. This fruit rich in many alkaloids, flavonoids, glycoside, saponins, tannins and phenols with rich nutritive values. Plant also has antibacterial, laxative, hepatoprotective antifungal, anti-giardial, antimicrobial, anti-inflammatory and analgesic properties. The fruit is used in strengthening, cooling effect, aphrodisiac and bowels astringent, expectorant, diuretic, also in blood purifier etc. Roots and leaves have anti-inflammatory, analgesic activities and also a antimicrobial activity. L-citrulline is almost lacking from the natural foods and watermelon being a notable exception**.**

## MATERIALS AND METHODOLOGY

### MATERIALS

**Tab 1: List of material used for preparation of Hydrogel beads.**

| S. No. | MATERIAL | COMAPANY NAME |
|---|---|---|
| **1** | Scopolamine | |
| **2** | Sodium Hydroxide | Sigma-aldrich |
| **3** | EDTA (ethylene diamine tetra acitic acid) | SD fine chem ltd |
| **4** | Hydrogen peroxide 30%, ExcelaR | Fisher chemicals |
| **5** | Potassium dihydrogen phosphate GR(anhydrous) 99% + | Chemikabiochemika |
| **6** | DTNB(5,5-dithiobis (2-nitro benzoic acid) | Himedia chemicals |
| **7** | Thiobarbituric acid (TBA) | Himedia chemicals |
| **8** | Hydrochloric acid | Qualigens fine chemi |
| **9** | Pyrogallol AR , 98.5% | Himedia chemicals |
| **10** | Tris(hydroxymethyl)aminomethane AR | SDFCL |
| **11** | Disodium hydrogen phosphate | SD fine chem. Ltd. |

| | | |
|---|---|---|
| **12** | Folincoicalteau phenol reagent | Fisher scientific |
| **13** | Trypan blue dye, 1-chloro-2, 4 dinitrobenzene (CDNB) | Chemika |
| **14** | Sulphanilamide AR | Himedia |
| **15** | Sodium nitrite | Sigma-aldrich |
| **16** | Phosphoric acid | Qualigens fine chemi |
| **17** | N-(1-naphthyl) ethylene diaminedihydrochloride | ChemikanBiochemika reagents |
| **18** | Glutathione | Lobachemie |
| **19** | NADPH(Nnicotinamideadninedineuclotidephosphateoxidase) | Himedia chemicals |

**1. Preparation of extract**

The sun dried of Citrullus lanatus was grounded, powder and 500g grounded seeds first Defatting with pet ether then extracted 95% ethanol for 10 hrs in asoxhlet extractor. The filtrate collected and evaporated to dryness pressure to yield the dry extract using rotavapour (decibel instrument).

**2. Experimental design**

**Tab 2: Experimental Design.**

| Group | No. of rats | Group name | Route, Dose |
|---|---|---|---|
| **I** | 5 | Normal Control | D.W (2ml/kg, I.P) |
| **II** | 5 | Scopolamine treated | (1 mg/kg, I.P.) |
| **III** | 5 | CLSE + Scopolamine | (150 mg/kg, oral.)+(1 mg/kg, I.P.) |
| **IV** | 5 | CLSE + Scopolamine | (300 mg/kg, oral.)+(3 mg/kg, I.P.) |
| **V** | 5 | CLSE (Per se) | (300 mg/kg, oral.) |

**a. Dosing Schedule**

- All the animals were divided into 5 groups (each group have 5 rats)
- First group served as a normal control treated with Distilled water 1 mg/kg (i.p.)
- Second group served as Toxic group (Scopolamine) with dose 1mg/kg (i.p.)
- Third group served as treatment group with dose of 150 mg/kg (oral)+ 1m/kg (I.P.)
- Fourth group served as treatment group dose of 300 mg/kg (oral) + 1mg/kg (i.p)
- Fifth group served as therapeutic dose as per se group of 300 mg/kg (oral)

**b. Indication of Amnesia**

Scopolamine (1 mg/kg), a muscarinic receptor antagonist, was dissolved in normal saline (0.9 % NaCl) and administered intraperitoneally in a volume of 1ml/kg body weight, Rats were subjected to behavioural testing 5 min after scopolamine injection.

**c. EVALUATION PARAMETERS**

**i. Behavioral parameters**

**ii. Morris water maze test**

**d. Thiobarbituric Acid Reactive Substances (TBARS)**

**Reagents**

- 0.8 % TBA solution
- 30 % TCA solution
- Standard TEP reagent

➢ **Catalase**

**Reagents**

- Potassium phosphate buffer (50 mM; pH 7.4)
- Hydrogen peroxide 30 % (19 mM/L)

**e. Protein Estimation Using Folin Reagent**

**Principle**

Protein reacts with Folin Ciocalteau reagent to give a coloured complex, the absorbance of which determined spectrophotometrically at 750 nm. The reaction involves two steps.

- Biuret test
- Reduction of Folin reagent

**f. Histopathological Studies**

**Procedure:** The following steps are involved in the Haematoxylin and Eosin staining:

The slides are observed under the microscope and the photographs are taken subsequently.

**RESULTS**

**1. Effects of Citrullus Lanatus Seed Extract on Morris water maze test in rats.**

The data provided in the given table where (n=5) in respectively group and all the values were expressed as mean ± S.E.M. data were analysed by ANOVA followed by Dennett's Multiple Comparison Test. From this study, we observed that on first day scopolamine treated group showed increase in latency time on final day from 26.4±0.2703 to 52.6±0.366, which was significant. The extract treatment group i.e 150 and 300 mg/kg showed none significant, when associated with scopolamine frozen group. On 15$^{th}$ day scopolamine treated group showed increase in latency time from 15.8± 0.200 to 36.4±0.454, which was

significant. The extract treatment group i.e. 150 and 300 mg/kg showed a reduction in latency time from 36.4±0.454 of scopolamine treated group 19.0±0.114 and11.0±0.966.

CLSE = *Citrullus Lanatus* Seed Extract.

SEM = Standard Error Mean

**2. Effects of Citrullus Lanatus Seed Extract on Elevated plus maze test in rats**

The data provided in the given table where (n=5) in each group and all the values were expressed as mean ± S.E.M. data were analysed by ANOVA followed by Dennett's Multiple Comparison Test. From this study, we observed that scopolamine treated group showed increase in number of entries in open arm from 6.6±0.503 to 36.4±0.707, which was significant. The extract treatment group i.e. 150 and 300 mg/kg showed a reduction in entries in open arm from 36.4±0.707 of scopolamine treated group 10.6±0.691 and 5.8±0.417.

CLSE = Citrullus Lanatus Seed Extract

SEM = Standard Error Mean

All values were expressed as Mean ± S.E.M. of 5 rats in each group.

**P<0.01, when compared with normal control group (I).

##P<0.01, when compared with scopolamine treated group (II).

**3. Effects of *Citrullus Lanatus* Seed Extract on motor coordination using grip strength meter in rats**

Table 5.3 Effects of Citrullus Lanatus on motor coordination using grip strength

The data provided in the given table where (n=5) in each group and all the values were expressed as mean ± S.E.M. Data were analysed by ANOVA followed by Dennett's Multiple Comparison Test. From this study, we observed that scopolamine treated group showed decrease in grip strength from 0.7516±0.001 to 0.3058±0.008, which was significant. The extract treatment group i.e. 150 and 300 mg/kg showed an increase in entries in open a grip strength from 0.3058±0.008 of Scopolamine treated group 1.1204±0.006 and 1.1704±0.009.

CLSE = Citrullus Lanatus Seed Extract

SEM = Standard Error Mean

All values was expressed as Mean ± S.E.M of 5 rats in each group.

**P<0.001, when compared with normal control group (I).

##P<0.001, when compared with Scopolamine treated group (II).

**4. Effects of *Citrullus Lanatus* seed extract on Acetyl choline esterase (AchE) level in rats**

Table 5.4 Effects of Citrullus Lanatus seed extract on AchE

The data provided in the given table where (n=5) in each group and all the values were expressed as mean ± S.E.M. data were analysed by ANOVA followed by Dennett's Multiple Comparison Test. From this study, we observed that scopolamine treated group showed decrease in grip Acetylcholine level from 0.21906±0.01155 to 0.65882±0.01373, which was significant. The extract treatment group i.e. 150 and 300 mg/kg showed an increase in entries in open a grip strength from 0.65882±0.01373 of Scopolamine treated group 0.5545±0.01287 and 0.3652±0.01505.

CLSE = Citrullus Lanatus Seed Extract

SEM = Standard Error Mean

All values was expressed as Mean ± S.E.M of 5 rats in each group.

**P<0.01, when compared with normal control group (I).

##P<0.01, when compared with Scopolamine treated group (II).

**5. Effects of *Citrullus Lanatus* seed extract on Catalase and TBARs acid activity in rats**

Table 5.5 Effects of Citrullus Lanatus seed extract on Catalase & TBARs acit Activity.

The data provided in the given table where (n=5) in each group and all the values were expressed as mean ± S.E.M. data were analysed by ANOVA followed by Dennett's Multiple Comparison Test. From this study, in case of catalase, we observed that Scopolamine treated group showed decrease catalase level from 9.644±0.07474 to 4.6034±0.05012, which was significant. The extract treatment group i.e 150 and 300 mg/kg showed an increase catalase level from 4.6034±0.05012 of Scopolamine treated group 5.746±0.06875 and 7.0164±0.05611. In case of TBARs, we observed that scopolamine treated group showed increased TBARs level from 3.14±0.178 to 6.88±0.1668, which was significant. The extract treated group i.e 150 and 300 mg/kg showed a decrease TBARs level from 6.88±0.1688 of scopolamine treated group 4.46±0.1505 and 2.23±0.1424.

CLSE = Citrullus Lanatus Seed Extract

SEM = Standard Error Mean

All value was expressed as Mean ± S.E.M. of rats in each group.

**P<0.01, when compared with normal group (I).

##P<0.01, when compared with Scopolamine treated group (II).

### 6. Effects of Citrullus Lanatus seed extract on SOD and GSH activity in rats

Table 5.6 Effects of Citrullus Lanatus seed extract on SOD & GSH

The data provided in the given table where (n=5) in each group and all the values were expressed as mean ± S.E.M. data were analysed by ANOVA followed by Dennett's Multiple Comparison Test. From this study, in case of SOD, we observed that Scopolamine treated group showed decrease SOD level from 1.507±0.01 to 0.6954±0.05, which was significant. The extract treatment group i.e 150 and 300 mg/kg showed an increase SOD level from 0.6954±0.05 of Scopolamine treated group 1.2402±0.0.09 and 1.272±0.011. In case of GSH, we observed that scopolamine treated group showed decreased GSH level from 0.59182±0.01974 to 0.30742±0.00283, which was significant. The extract treated group i.e 150 and 300 mg/kg showed an increase GSH level from 0.30742±0.00283 of scopolamine treated group 0.42923±0.00605 and 0.49906±0.00958.

CLSE = Citrullus Lanatus Seed Extract

SEM = Standard Error Mean

**Graph Effect of *Citrullus Lanatus* seed extract on SOD and GSH activity in rats**

All value was expressed as Mean ± S.E.M. of 5 rats in each group.

**P<0.01, when compared with normal control group (I).

##P<0.01, when compared with Scopolamine treated group (II).

### 7. HISTORICAL STUDIES

Histopathology of brain section from different treatment groups stained with haematoxylin and eosin 10x.

a = Normal Control (NC)

b = Scopolamine 1mg/kg

c = Scopolamine+CLSE100 mg/kg

d = Scopolamine+CLSE300 mg/kg

e = Per Se (300 mg/kg)

### CONCLUSION

The result suggested that Flavonoid, L- Citrullin, phenolic compound, Vit C present in extract might be neuroprotective no- tropic or memory enhancement activity. The effect may be due to AChE, antioxidant effect or increase cholinergic transmission. The doses in this show some increase antioxidants activity. Further analysis may be required to find out the mechanism behind this no tropic activity.

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