Ameliorative Effect of Dried Seed Extract of *Prunus dulcis* (Mill.) D.A. Webb var. amara (DC.) on Scopolamine-induced Memory Impairment in Rats

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ABSTRACT

Present study includes the ameliorating effect of dried extract seed of *Prunus dulcis* (Mill.) D.A. Webb var. amara (DC.) on Scopolamine hydrobromide (ScHBr) induced memory impairment in rats, cholinesterase activity and oxidative stress. Cognitive disorders include various factors as amnesia, difficulties in attention and Alzheimer’s disease (AD) and other neurologically disorders. Basic mechanism of neuro related disorder underlies with neuroapop忿s. The term Neuroapop忿s means dead of neurons, leads to dementia, abnormal behaviour, psychological changes which may even leads to death of the individuals. Characteristics features of this disease include irregularity in learning and memory, deposition of senile plaques and neurofibillary tangles, elevated level of Acetylcholinesterase enzyme (AChE) and oxidative stress. Amnesia causing agent includes Scopolamine hydrobromide, which tends to increase AChE activity. Extract dose was administered at 250mg/kg and 500mg/kg of body weight of animals for consecutive 7th and 8th day for Elevated Plus Maze (EPM) and 10th and 11th day for Morris water maze (MWM) to the various groups of animals Standard Nootropic agents was used for this study is Piracetam at a dose of 120mg/kg of body weight. Summary Results indicate that extract of seed have potential to decrease transfer latency at 7th day and 8th day in the EPM task and transfer latency on acquisition day i.e 10th day and transfer latency (TL) on retention day on 11th day in Morris Water Maze. PDWA extract shows decreased the activity of cholinesterase in brain and also exhibited improve antioxidant defence system leads to decrease level of Thiobarbituric acid reactive substance TBARS which is indication of diminished oxidative stress and improve cognitive function.

Key words: Scopolamine hydrobromide (ScHBr), *Prunus dulcis* (Mill.), (PDWA), Amnesia, Acetyl cholinesterase (AChE) Thio Barbituric Acid Reactive Substances (TBARS), Cognition.

INTRODUCTION

Cognitive disorders include various factors as amnesia, difficulties in attention and Alzheimer’s disease and other neurologically disorders. Basic mechanism of neuro related disorder underlies with neuroapop忿s. The term Neuroapop忿s means dead of neurons, leads to dementia, abnormal behaviour, psychological changes which may even leads to death of the individuals.[1]

Neurodegenerative disorders include various disease like Parkinson’s, Huntington’s disease and also precipitates Alzheimer’s disease (AD). AD is characterized by the deficits in memory and impairment of reasoning process.[2]

Various cause for improper function of cognition in healthy individuals include, degeneration of neurons in the brain, inflammation of neurons, change in receptor activity, improper regulation in neuro-transmitter like serotonin, adrenaline, acetylcholine, dopamine and NMDA[3] and excess production of Reactive oxygen species and imbalance in quenching of this free radicals.[4]

Acetylcholine a neurotransmitter shown prominent effect in cognition behaviour, which is proved by number of experimental and clinical data.[3] Various neurotransmitter is present in our brain among which Acetylcholine ACh have selected role for its formation of hippocampal, which serve as substrate for learning and memory function.[6-8]

For improvement of memory in the rat, a theory proposed from large number of clinical data is to cholinergic agonist and inhibitors of AChE, which tends to increase level of ACh in the synaptic cleft, where as medication based on Anti-cholinergic mainly impair learning and memory in a number of tasks.[9-13]

Muscarinic receptor is divided into three types i.e. M-1, M-2 and M-3 type out of which for learning and memory mainly M-1 receptor is responsible. This is mainly present at hippocampal, pre frontal and frontal regions.[14] Reduction in cholinergic receptor in hippocampal, pre frontal, cortex region and frontal regions leads to diminish activity of learning and memory capacity of brains. Mechanism for Neurodegenration are increase oxidative stress i.e production of Reactive Oxygen Species (ROS), superoxide, hydroxyl and perhydroxyl. These free radical frequently damage surrounding tissue and their products. This damaging property is known as oxidative damage and all the consequence which provide protection against this radicals known as antioxidants.

Damaging property of free radicals include disruption in myelin sheath in neurons, DNA damage, damage in lipid bi-layer of unsaturated fatty acids and plasma lipoproteins which leads to formation of high level

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of peroxides, dialdehyde level increases and also leads to disruption in tertiary and quaternary structure of protein and other molecules. It may also cause mutations, cancer, autoimmune disease and atherosclerosis. Over production of free radical also cause apoptosis of the cells. A.D shows occurrence of Neuro fibrillary tangles, Senile plaques, Amyloid proteins and tau-proteins precipitation is a clinical signs for neuronal apopototic pathway. In A.D all these factors are mainly showns in the area of lore brains, which serves as for learning, memory and other intellectual property.

Prunus dulcis (Mill.) D.A. Webb var. amara (DC.) is a subspecies of bitter almonds which is a wonderful herb that cures several diseases. Seed possess antioxidant properties, neuroprotective action, antistress, lipid lowering, laxative and immune-stimulant activity. Pharmacological uses of apricot seeds are generally in the healing and cure carcinoma like hepatocellular carcinoma and lung carcinoma in mentioned in well-known treatise system of various countries like China. Previously isolated constituent of the plant are palmitic acid, glutamic acid, glycoside are amygdalin, prussic acid, arginine although infusion of Prunus dulcis (Mill.) D.A. Webb var. amara (DC.) seed had been use for treatments of neuroprotective as a holistic medicine. In Ayurvedic texts kernels of the species of Prunus dulcis (Mill.) D.A. Webb var. amara (DC.) have been widely used as medahyarasayana and widely used as folklore practices.

MATERIALS AND METHODS

Plant Identification

The seed of bitter almonds is purchased from local market of Prayagraj, Uttar Pradesh. The authentication and identification of the plant specimen i.e. Prunus dulcis (Mill.) D.A. Webb var. amara (DC.) is done by Botanical survey of India office of the Scientist-E Central Regional Centre, 10, Chatham Lines, Allahabad-211002, India.

Plant Extract

The collected seeds were firstly peeled off, then with the help of blender convert it into powdered form, about 200 gm of powdered was subjected it into defatting with the help of petroleum ether and then subjected to filter with the help of muscelin cloth and then filtrate cake was subjected to dried at 50 degree for 10 min in hot air oven in order to remove moisture and make it dry. Take the defatted powdered and then subjected to extract with the help of soxhlet apparatus using mixture of 40% methanol+ 20% ethanol+ 40%water and the resulting obtained solution was placed in water bath (45 degree C).

Animals

Animals were purchased from CDRI (Central drug research institute) animal house having weight ranges in between 150-210 gm and 10-12 weeks old of albino wistar rats., for our research purpose. Storage condition maintained at 24 ± 2°C conditions is maintained. During research protocol diet was pellet and ad libitum water. Accommodation of the experimental animals were kept in isolation specified area in animal house at-least before the 14 days prior to start the protocol for the experiment, which is approved by I.A.E.C Institutional Animal Ethics Committee having. Registration-Number is UIP/IAEC/ DECEMBER-2019/12).

Drugs and Chemicals and Experimental design

Scopolamine hydrobromide 98% purchased from Yarrow Chem. Products (Mumbai Standard drug piracetam was obtained as a gift sample).

Experimental design

The alcholic extract of seed of Prunus dulcis (Mill.) D.A. Webb var. amara (DC.) give at a dose of 250mg/kg and 500mg/kg of the weight of the rats. For making solution of the extract we vehicle of this solution is distilled water. Experimental animals were classified as randomly into five groups and total 30 experimental animals were taken in which, each group receive six animals;

Group 1. Positive Control Group: Animals belonging to this group received vehicle only.

Group 2. Negative Control Group: Animals belonging to this group received Scopolamine hydrobromide (1mg/kg, b.w., i.p Intraperitonal injection).

Group 3. Experimental Group-I: Animals belonging to this group received Test drug (250mg/kg, b.w., p.o) at low dose with scopolamine hydrobromide (1mg/kg, b.w., i.p).

Group 4. Experimental Group-II: Animals belonging to this group received Test drug (500mg/kg, b.w., i.p) at higher dose with scopolamine hydrobromide (1mg/kg, b.w., i.p).

Group 5. Standard drug treated Group: Animals belonging to this group received standard drug Piracetam (120mg/kg, b.w.) with scopolamine hydrobromide (1mg/kg, b.w., i.p).

Administration of Test and Drug compounds

Administration of ScHBr (1mg/kg, b.w., i.p) was 60 min before the acquisition trials in group 3,4 and 5. PDWA extract at a dose of 250 and 500mg/kg were given for subsequent days respectively and acquisition trial was carried out 60 min after the last dose. Standard Nootropic medication-Piracetam was administered by using normal saline solution at dose of 1mg/kg. Standard drug, Plant extract was administered 30 min before the conducting of the experiments.

Elevated plus maze

EPM (Elevated plus maze) behavioural model principally works as giving incentive from the outside environment not as inside ones. EPM consist of two arms open arm and closed arm having dimension about 48cm x 10cm. For induction of anxiety in rats EPM was elevated 50cm.Parameters studied in EPM were Transfer Latency and Memory retention. Retention latency is inspected 24 hr after transfer latency.

Morrissy water maze

Maze have unique dimension i.e. 50 x 120cm and water filled upto 30cm. Pool is divided into four compartment N, E, W and South. Removable Platform (29 X 8cm) kept at NE North East zone. Water is made opaque colour so that central platform become hidden. The basic principal is to find platform and climbed on it so that rat can avoid unnecessary swimming. On the first day rats were kept at SW SOUTH WEST quadrants and allow it to find platform within 120sec. Practice session is held for 4-5 days. In such case if rats did not find platform, the researcher guide towards the platform and assigned as transfer latency. For better activity minimum number of trails per day is about 5 and continues to be 5-7 days.

Biochemical analysis

Method for performing Euthanesia and Brain – homogenation.

After performing retention trials, the animals used during protocol, were gently sacrificed. By the means of decapitated ones. After which the brain was removed from the skull. With the help of Teflon, Homogenizer the excluded brain was homogenized in a medium containing (phosphate’s
Various animal group i.e. control group, ScHBr treated group, PDW A and Piracetam (120mg/kg) + ScHBr 1mg/kg were evaluated at 10th day. The ScHBr treated group showed significantly increased in ITL value on 10th day i.e. acquisition trial day as well as subsequent day 11th day i.e. retention trial day as compared to control group, which shows cognitive dysfunction. ITL value were increased at (P<0.05). Extract of PDWA at a dose of 250mg/kg and 500mg/kg showed decreased TL on 7th day as compared to ScHBr treated group. Statistically significant result were obtained i.e. (P < 0.005). In the next subsequent day we measure retention trial at 8th day of the experimental protocol at extract dose of 250mg/kg and 500mg/kg showed statistically significant (p<0.05) as compared to ScHBr treated group with respect to retention trials. Standard Nootropic medication i.e. Piracetam also exhibit decrease ITL as compared to ScHBr treated group at a significant (p<0.05) as shown in Figure 1 and in Figure 4 showed movement of rat in elevated plus maze, which was further analyse by motion tracking software i.e. Any maze.

Morris water maze
Various animal group i.e. control group, ScHBr treated group, PDWA (250mg/kg ) + ScHBr 1mg/kg, PDWA (500mg/kg) + ScHBr 1mg/kg, Piracetam (120mg/kg) + ScHBr 1mg/kg were evaluated at 10th day. The ScHBr treated group showed significantly increased in ITL value on 10th day i.e. acquisition trial day as well as subsequent day 11th day i.e. retention trial day as compared to control group, which shows cognitive dysfunction. ITL value were increased at (p<0.05). Extract of PDWA at a dose of 250mg/kg and 500mg/kg showed decreased ITL on 10th day as compared to ScHBr treated group. Statistically significant result were obtained i.e. (p<0.05). In the next subsequent day we measure retention trial at 11th day of the experimental protocol at extract dose of 250mg/kg and 500mg/kg showed statistically significant (p<0.05) as compared to ScHBr treated group with respect to retention trials. Standard Nootropic medication i.e. Piracetam also exhibit decrease ITL as compared to ScHBr treated group at a significant (p<0.05). Extract dose of PDWA and Piracetam shows increase time spent in targeted quadrant as compared to ScHBr treated group as shown in Figure 2 and in Figure 5 showed movement of rat in morris water maze, which was further analyse by motion tracking software i.e. Any maze.

AChE level in the rat brain and level of acetylcholine.
ScHBr treated animals shows decrease level of acetylcholine and increase level of the AChE activity in the brain as compared to control group. With the use of extracts of seeds of Prunus dulcis (Mill.) D.A. Webb var. amara (DC.) at 250 mg/kg and 500 mg/kg, it had been shown that there is increase level of acetylcholine and decrease activity of AChE activity as compared to ScHBr treated group as significant (p<0.05) statistically. Standard Nootropic medication i.e. Piracetam also exhibit decrease AChE activity, hence leads to increase level of acetylcholine as compared to ScHBr treated group at a significant (p<0.05) as shown in Figure 3.

Estimation of TBARS levels in the brains
ScHBr treated animals shows increase level of T.B.A.R.S in the brain as compared to control group, with the use of extracts of seeds of Prunus dulcis (Mill.) D.A. Webb var. amara (DC.) at 250 mg/kg and 500 mg/kg, it had been shown that there is decrease level of T.B.A.R.S as compared to scopolamine treated groups at a significant (p<0.05). Standard Nootropic medication i.e. Piracetam also exhibit decrease TBARS level as compared to ScHBr group (p<0.05) as shown in Figure 6.
Figure 1: Effect of alcoholic extract of PDWA on TL and RL on EPM. N=6 and values are expressed as mean± SEM. One way ANOVA followed by Dunnet’s test, *p < 0.05, as comparing with control, *p< 0.05 compared with ScHbr treated group, *p< 0.05 compared with control group.

Figure 2: Effect of alcoholic extract of PDWA on TL and RL on MWM. N=6 and values are expressed as mean± SEM. One way ANOVA followed by Dunnet’s test, *p < 0.05, as comparing with control, *p< 0.05 compared with ScHbr treated group, *p< 0.05 compared with control group.

Figure 3: Effect of alcoholic extract of PDWA level of AChE. N=6 and values are expressed as mean± SEM. One way ANOVA followed by Dunnet’s test, *p< 0.05, as comparing with control, *p< 0.05 compared with ScHbr treated group.

Figure 4: Analysis of rat movement by motion-tracking software on Elevated plus maze paradigms. Q1 = Control group, Q2= PDWA (500 mg/kg), Q3= Piracetam (120 mg/kg).

Figure 5: Series A represent Standard drug of Piracetam treated rats, series B represent dose of extract of PDWA at 500mg/kg body weight, series C represent Control treated groups.

Figure 6: Effect of alcoholic extract of PDWA on level of the TBARS. N=6 and values are expressed as mean± SEM. One way ANOVA followed by Dunnet’s test, *p< 0.05, as comparing with control, *p< 0.05 compared with ScHbr treated group. Effect of alcoholic extract of PDWA on level of AChE. N=6 and values are expressed as mean± SEM.
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(Mill.) D.A. Webb var. amara (DC. To evaluate its pharmacological property in cognitive impairments disease. Symptoms for cognitive impairments include neuro-apoptotic, neuro-inflammation, oxidative stress, dementia, amnesia and decrease level of Acetylcholine, which have primarily role in learning and reasoning process.

For standardization of alcholic extract of Prunus dulcis (Mill.) D.A. Webb var. amara (DC.) seeds various parameters has been performed like presence of unwanted substance and also determine its various extractive value and pharmacognostical parameters.

For inducing amnesia in experimental rats widely proposed model has been used i.e. Scopolamine – hydrobromide. ScHbr have anti-muscarinic activity, which leads no net binding of Acetylcholine to its receptors and increase the activity of anti-cholinesterase activity leads to impairment in learning and memory behaviour.

Two behavioural model has been used to check memory in rats include elevated plus paradigms and morris water maze paradigms. Dose of alcholic extract of seeds of Prunus dulcis (Mill.) D.A. Webb var. amara (DC.) on scopolamine induced rats had been performed at 250mg/kg body weight and 500 mg/kg body weight. For induction of amnesia and dementia ScHbr is used at a dose of 1mg/kg body weight. Alzheimer's and other neural related disorder is mainly characterized by loss of memory, dreamy- state, loss in attention, attention deficient patients having increase level of their activity etc. If such disease are not treated, then its leads to death. Unfortunately the exact treatment for such disorder are not yet discovered. So an effort has been taken to investigate such disease and treat them. Prunus dulcis (Mill.) D.A. Webb var. amara (DC.) contains poly-phenolic compounds, amygdalin and other chemical constituents. For evaluating such disorders various animals models has been used, the extract of seed at a dose of 250mg/ kg body weight and 500mg/kg of body weight reduced transfer latency at acquisition trial and retention trial at 10th and 11th day as compared to scopolamine treated group and have potential to reverse the effect of scopolamine given to the drug. Hence the extract of PDWA shows increase in cognitive function. Further biochemical parameters has been performed which shows decrease level of cholinesterase activity in the brain shows improve the activity of acetylcholine in the brain, which is a major neurotransmitter for learning associated process and decrease levels of TBARS levels in the brain shows improve level of antioxidant system in the brain which leads to decrease the level of oxidative stress leads to healthy activity of neuron in the brain and showed that this drug can be used for future treatment in cognitive impairment disease.

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CONFLICT OF INTEREST

The authors declares no conflict of interest in the content and writing of this manuscript.

ABBREVIATIONS

SchBr: Scopolamine hydrobromide; AChE: Acetylcholinesterase; EPM: Elevated Plus Maze; MWM: Morris water maze; AD: Alzheimer's disease; TBARS: Thiobarbituric acid reactive substance; PDWA: Prunus dulcis (Mill.) D.A. Webb var. amara (DC.); NMDA: N- methyl-D-aspartate; AcH: Acetylcholine; ROS: Reactive Oxygen Species; DNA: Deyoxyribonucleic acid; CDRI: Central drug research institute; IAE: Institutional Animal Ethics Committee; Bw: Body weight; IP: Intraperetional injection; NE: North east; SW: South west; Cm: Centimetre; TBA: Thio barbituric acid; MDA: Malondialdehyde; SDS: Sodium dodecyl sulphate; UV: Ultraviolet; ANOVA: Analysis of Variance.

REFERENCES

Alzheimer's and other neuro related disorder is mainly characterized by loss of memory, dreamy-state, loss in attention, attention deficient patients having increase level of their activity etc. If such disease are not treated, then its leads to death. Unfortunately the exact treatment for such disorder are not yet discovered. So an effort has been taken to investigate such disease and treat them. Prunus dulcis (Mill.) D.A. Webb var. amara (DC.) contains poly-phenolic compounds, amygdalin and other chemical constituents. For evaluating such disorders various animals models has been used, the extract of seed has shown to decrease latency times in both the maze i.e. elevated plus maze and morris water maze. Further biochemical parameters has been performed which shows decrease level of acetyl-cholinesterase enzyme activity which leads to increase concentration of acetylcholine in the brain and decrease levels of TBARS levels, shown that this drug can be used for future treatment in cognitive impairment disease.