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Neuroprotective activity of Phoenixsylvestris against colchicine induced neuroinflammation and cognitive deficits in rat

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KEYWORDS
Neuroinflammation, Cognitive deficits, Phoenix sylvestris, Colchicine, Acetylcholinesterase.

ABSTRACT:
Introduction: Colchicine induced neuro-inflammation and cognitive deficits can be cured by the Phoenix sylvestris.

Objectives: The present study was designed to evaluate the neuroprotective effect of Phoenix sylvestris against colchicine induced Neuroinflammation and cognitive deficits in rats.

Methods: Neuroprotective activity was carried out by colchicine induced Neuroinflammation and cognitive deficits on rats. Test group animals administrated with ethanol extract Phoenix sylvestris (EPS) at dose of 150 mg/kg, 300 mg/kg, and 500mg/kg for 28 days. Neurobehavioral tests like Morris water maze, Elevated plus maze, Radial arm maze were performed. Inflammatory mediators like TNF-∝, IL-6 were assessed. Followed by biochemical parameter such as LPO, GSH, SOD, CAT, total thiols and ACh were assessed and histopathology of brain tissue were studied on the final day of the experiment.

Results: EPS shows a dose dependent neuroprotective activity with significant decrease in time latency of Morris water maze, elevated plus maze and radial arm maze in EPS treated group compared to colchicine induced control group. Inflammatory mediators showed decrease in TNF-α, increase in IL-6. Biochemical estimations showed significant decreases in LPO and increased in SOD, CAT, GSH, total thiols & Ach level in the EPS treated group as compared with colchicine induced control group. Molecular docking results showed main constituents of Phoenix sylvestris could interact with TACE and AChE.

Conclusions: EPS treatment could reduce neuroinflammation and preventing the development of cognitive deficits. In conclusion, the present study suggests that administration of EPS possesses neuroprotective activity against colchicine induced Neuroinflammation and cognitive deficits.

Introduction

Neuroinflammation initiated in response to a range of cues, inclusive of infection, tense Genius injury, poisonous metabolites, or autoimmunity. In the central fearful device, together with the intelligence and spinal cord, microglia are the resident innate immune cells that are activated in response to these cues. The CNS is usually an immunologically privileged site due to the fact peripheral immune cells are typically blocked via the blood-brain barrier (BBB), a specialized shape composed of astrocytes and endothelial cells. However, circulating peripheral immune cells might also surpass a compromised BBB and come upon neurons and glial cells expressing essential histocompatibility complicated molecules, perpetuating the immune response. Although the response is initiated to defend the central frightened system from the infectious agent. The impact might also be poisonous and enormous

infection as properly as similar migration of leukocytes through the blood-brain barrier. Neuroinflammation is extensively viewed as chronic, as antagonistic to acute, irritation of the central worried system. Acute infection typically follows damage to the central anxious machine immediately and is characterized by means of inflammatory molecules, endothelial cell activation, platelet deposition, and tissue edema. Chronic irritation is the sustained activation of glial cells and recruitment of different immune cells into the brain. It is a continual infection that is normally related to neurodegenerative diseases. Inflammation may additionally be an essential mechanism underlying dementia and cognitive decline in the elderly. Inflammation has been implicated in the neuropathological cascade main to the improvement of Alzheimer's disorder and different frequent types of dementia in later life. These observations have led to observational epidemiological learn about to outline the affiliation of systemic and talent inflammatory markers on cognitive impairment and dementia. Furthermore, scientific trials have been carried out to higher elucidate the feasible function of nonsteroidal anti-inflammatory drugs (NSAIDs) in the prevention or slowing of the development of Alzheimer's disease. The function of infection in neurological problems is an increasing number of recognized. Inflammatory strategies are related to the etiology and medical development of migraine, psychiatric conditions, epilepsy, cerebrovascular diseases, dementia, and neurodegeneration, such as viewed in Alzheimer's or Parkinson's disease. Both central and systemic inflammatory moves have been linked with the improvement of intelligence diseases, suggesting that complicated neuro-immune interactions should contribute to pathological modifications in intelligence throughout more than one temporal and spatial scale (Ebert SE, Jensen P 2019).

Colchicine is an alkaloid derivative isolated from *Colchicum autumnale* and *Gloriosa excellent* plants of the lilly family that binds irreversibly to the microtubules of neurons and promotes de-polymerization of microtubules leads to inhibit their assembly. These consequences in the impaired intracellular trafficking of neurotrophic factors, synaptic loss and multiplied axonal excitotoxicity (Mandelkow EM, Stamer K 2003).

Phoenix sylvestris is native to India and southern parts of Pakistan commonly recognized as Indian date. One traditional plant, *Phoenix sylvestris*, is broadly acknowledged as Wild date palm. The plant contain quercetin, apigenin, luteolin and glucans. It is pharmacologically used as antimicrobial activity, antioxidant activity, antiulcer activity, antidiarrheal activity, antinociceptive, anti-mutagenic activity, anticancer activity, anti-obesity activity, hematopoietic activity& hemolytic activity. Roots are used in diuretic activity and analgesic activity. Seeds are used in anti-inflammatory activity. Leaves are used in antidiabetic activity. In proceedings (Sandhiya, Ratna Nirmala2021) (Jain P, Jain S).

The aim of present study is to evaluate the neuroprotective effect of *Phoenix sylvestris* against Colchicine induced Neuroinflammation and cognitive deficits in rats.

Materials and Methods

Chemicals and reagents

Colchicine (Ref no.C3915-1G), TNF-alpha, IL-6, Epinephrine (Ref no.E4250-10G), 2-thiobarbituric acid(TBA) (Ref no.T5500-25G), Ellman’s reagent or DTNB (5-5’- Dithiobis (2-nitrobenzoicacid) (Ref no.D8130-1G) all were purchased from Sigma-Aldrich Co. Acetylthiocholineiodide(Refno.RM770-1G)waspurchasedfromHi-Media,Follen’sreagentwaspurchased from Spectrochem Pvt. Ltd. Mumbai &Trichloroacetic acid (TCA), All otherchemicalswere of the Analytical gradeare commerciallyavailable.

Plant material

In the Present study *Phoenix sylvestris* fruits were purchased from the market of Bagalkote, Karnataka, India during the month of January 2022. Fruits were identified and authenticated in the Department of Botany, Basaveshwar Science College, Bagalkote, Karnataka and Voucher specimen (BSc/Bvt/2022/05) was deposited in the herbarium of the same college for further future reference. All the fruits were thoroughly cleaned, chopped to fine pieces and shed dried at room temperature for complete drying. Finally, fine pieces were subjected to pulverize to get coarse powder. The coarse powder was stored in air tight polyethylene container before extraction. (The coarse powder of fruits were subjected to maceration process for 48hour

initially with petroleum ether at60-80^{0}C followed by ethanol at 64-65.5^{0}C.) After solvent was distilled off from the residue of the extraction and excess solvent was completely removed by allowing it to evaporate at room temperature to get concentrated extract then completely dried by using lyophilizer (Mini Lyotrap, LTE Scientific Ltd, Great Britain) and orange colour solid mass was stored in air tight container under refrigeration. The obtained extract was104gm used for Neuroprotective activity.

Acutetoxicitystudy

The female Swiss Albino mice (25-30mg) were administered with limit dose the acute toxicity was studied according to the OECD guidelines 425. Limit dose of 2000mg/kg (p.o.) was administered and they were observed for behaviour and other signs of toxicity, such as excitation, tremors, twitches, motor coordination, righting reflex and respiratory changes for 4hrs and monitored up to 14days. No mortality was occurred with higher limit dose. So the doses were selected as 150mg/kg, 300mg/kg&500mg/kg.

Animals

The Sprague-Dawley rats of either sex (220-270 gm) were obtained from the central animal house of H.S.K College of Pharmacy and Research Centre, Bagalkote, Karnataka. The animals were housed at room temperature (28±2^{0}C) with 65±10% relative humidity for 12hrs dark and 12hrslight cycle and given standard laboratory feed and sufficient drinkingwater*adlibitum.*Thestudywereapprovedandconductedasperthenormsofthe InstitutionalAnimalEthicsCommitteeofH.S.KCollegeofPharmacy,Bagalkote(RefNo. IAEC/HSKCOP/Aug2021/PG2)andcarriedoutinaccordancewiththe CPCSEA Guidelines for the use and care of laboratory animals.

Experimental protocol for Neuroinflammation and cognitive deficits.

Sprague Dawley rats of either sex (220-270gm) were divided into five groups of 8 rats in each group and fed with drug orvehiclefor28 days and treated as follows.

Group I: Sham received 5µl of ACSF and 0.2% ascorbic acid by stereotaxic injection into the right lateral cerebral ventricle and vehicle orally for28days.

Group II: Control group received 5µl of Colchicine (15ụg/rat) in ACSF and 0.2%ascorbic acid by stereotaxic injection into the right lateral cerebral ventricle and vehicle orally for28 days.

Group III: EPS 150mg/kg group received 5µl of Colchicine (15ụg/rat) in ACSF and0.2% ascorbic acid by stereotaxic injection into the right lateral cerebral ventricle and ethanol extract of *Phoenix sylvestris* (150mg/kg, orally) for 28days.

Group IV: EPS 300mg/kg group received 5µl of Colchicine (15ụg/rat) in ASCF and0.2% ascorbic acid by stereotaxic injection into the right lateral cerebral ventricle and ethanol extract of *Phoenix sylvestris* extract(300mg/kg,orally) for28days.

Group V: EPS 500mg/kg group received 5µl of Colchicine (15ụg/rat) in ACSF and0.2% ascorbic acid by stereotaxic injection into the right lateral cerebral ventricle and ethanol extract of *Phoenix sylvestris* extract(500mg/kg,orally) for28days.

Surgery and intra cerebroventricular administration of colchicine

Animals weighing 220-270gm were anesthetized with Ketamine (75mg/kg, i.p.) and positioned in a stereotaxic apparatus in which the frame was previously calibrated and cleaned with 70% alcohol. The head was positioned properly in a frame and midline sagittal incision was made in the scalp and bregma was exposed. One hole was drilled in the skull for the placement of the injection cannula into the right lateral cerebral ventricles Co-ordinates for the intra-cerebro ventricular cannula implantation had been 0.8 mm posterior to bregma, 1.8 mm lateral to the sagittal suture and 3.6 mm below the cortical surface. Rats were infused with colchicine (15µg/rat) dissolved in 5µl of artificial cerebrospinal fluid (ACSF; inn M:NaCl147, KCl2.9, MgCl21.6, CaCl21.7 and dextrose 2.2), was slowly injected into the cannulated right lateral ventricle using a 25µl Hamilton syringe and the needle washed in place for 2-3 minutes for proper dispersal of the drug from the tip of the syringe. The scalp used to be then closed with a suture. Iodine or Betadine

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solution was applied to the surgical area with help of cotton in order to prevent sepsis. Animals were housed individually in a separate cage with soft bedding. Special care of the animal was taken during postoperative period to provide food and sufficient water inside the cage of rats Sham group were subjected to the same surgicalprocedureandreceivedartificialcerebrospinalfluid .Postoperatively, rats were kept in separate cages and monitored (Anil Kumar, Samrita Dogra, and Atish Prakash2010).

Determination of the content of quercetin in Ethanol extract of *Phoenix sylvestris* by HPLC.

Sample preparation

Standard stock solution preparation: 10mg drug was accurately weighed and put in to 10ml volumetric flask containing 5ml of diluents and sonicated for 10min then the volume was adjusted with diluents up to the mark. Sample solution concentration 10µg/ml was prepared from above stock solution and diluted with diluents and filtered through 0.22µ Millipore membrane filters and injected in HPLC system.

Preparation of sample: 10mg EPS was accurately weighed and made a sample solution concentration as above.
RP-HPLC Method: RP-HPLC instrument equipped with SPD-10AVP-vis detector (Shimadzu, Japan). An auto-sampler, Nucleodur, C18 (4.6×250mm i.d, 5µm particle size) and an LC-solution software (Mathew R, Varkey J2021) and (Nag M, Kar A 2020).

Molecular Docking studies

Molecular docking is considered as the “key and lock” hypothesis used to find the best fit orientation of ligand and protein. Phytochemicals from *Phoenix sylvestris* fruit extracts were selected for molecular docking study. The list of phytocompounds like Diethyl nitrosamine, 2, 3-Dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one, 4-methylcatechol, 2, 4-Di-tert-butylphenol& Diethyl Phthalate were further subjected to molecular docking studies.

The three-dimensional crystal structure of TNF-alpha converting enzyme (PDB ID:2fV5) and Acetylcholinesterase (PBD ID:4EY5)(Alam A, Tamkeen N 2018)downloaded from RCSB Protein data bank (PDB) water molecule in 2fV5 and 4EY5 were removed followed by addition of Kollaman's charges and polar hydrogen using AutoDock 1.5.6 tools (Morris GM, HUEY R 2012). The structures of ligands were drawn by using ChemDraw 16.0 and copied as SMILES. The Avogadro tool (Hanwell MD, Curtis DE, Lonie DC 2012) was used to generate the pdb file format of ligands with energy minimization. All structures were saved as pdb file format for input to AutoDock 1.5.6. The grid file was generated and saved as gpf file format. The Lamarckian Genetic Algorithm (LGA) was used to find the conformers with the lowest binding energies and the dpf file were generated to dock. Final docking task performed by using AutoDock. After completion of docking command file, the docked file was saved in dlg format and ready to analyse the binding interaction of ligand and receptors in different conformations. The highest binding energy of conformation of ligand was selected and visualized the interactions in the Biovia discovery studio client 2021(Trott O, Olson AJ 2010).

Neurobehavioral studies

Behavioral assessment

Morris Water Maze test

Spatial learning and memory were tested in a Morris water maze. The maze consisted of a Black circular pool (diameter 182 cm, radius 91 cm, height 75 cm) filled to a depth of 30 cm with water (25±2℃). On pre-surgical day, rats acquired habituation (exposure in water maze for 1 minute) in which there used to be no platform present. Then, on Days 0, 7, 14, 21 and 28 a circular platform (10 cm in diameter) used to be saved hidden 2 cm under the water level in the middle of one of the quadrants (The platform remained the identical role for the duration of all the sessions). At the opening of every session, a random sequence of 4 beginning poles, along the perimeter of the pool was once generated. All animals accompanied this sequence for that session. Each rat was once positioned in the water dealing with the wall at the begin vicinity and was once allowed 90 seconds to discover the hidden platform. The animal were once allowed for 20 seconds relaxation on the platform. The latency to attain the platform used to be recorded. If the rat were to be unable to locate the hidden platform, it were once lifted out and positioned on the platform for 20 seconds. The technique was once repeated for all the

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4 begin locations. Two classes of 4 trails every have been performed on the subsequent day. Following that, the platform was once eliminated and probe trail (without platform) was once carried out 4 hours later. Each rat used to be positioned in the pool at the same by randomly choose beginning pole. The swimming time spent in the target quadrant (which at first contained platform) of the pool was once measured (Anil Kumar, Samrita Dogra, and Atish Prakash. 2010) and (Adham R. Mohamed, Gehan YS2015).

Parameters to be measured in Morris Water maze are:
(a) Escape latency
(b)Target quadrant latency
(c)Probe trail

Elevated plus maze test

The elevated plus maze consisted of two opposite black open palms (50 X 10 cm) and crossed with two closed walls of the same dimensions with 40 cm high walls. The hands have been connected with a central square of dimensions 10 X 10 cm. The entire maze used to be elevated to a peak of 50 cm from the floor. Acquisition of memory used to be tested on day 0, before colchicines administration. Animal was once positioned individually at one end of the open arm facing away from the central square. The time taken by the animal to move from the open arm to the closed arm used to be recorded as the initial transfer latency (ITL). Animal was once allowed to discover the maze for 20 seconds after recording the ITL and then returned to the home cage. If the animal did not enter the enclosed arm within 90 seconds, it was once guided on the back into one of the enclosed arm and the ITL used to be given as 90 seconds. Retention of memory used to be assessed by placing the rat in an open arm and the retention latency used to be noted on Days 7, 14, 21 and 28 of ITL and used to be termed as the first retention switch latency (1st RTL), 2nd retention transfer latency (2nd RTL) and retention transfer latency (3rd RTL), respectively (Anil K, Samrita D2009).

Each animal was once placed at the centre of the maze facing one of the enclosed arms. During the 5 min test period, the number of entries in open arm, number of entries in enclosed arm and time spent in open arm, time spent in enclosed arm have been recorded. Entry into an arm was once defined as the point when the animals place all 4 paws onto the arm. After the test the maze was once carefully cleaned with a wet tissue paper (10% ethanol solution) (ZhaoY, MinyanD, WenzihiZ 2020).

Parameters to be measured in Elevated plus maze test are:

(a) Initial transfer latency
(b) Time spent in open arm
(c) Time spent in closed arm
(d) Number of entries in open arm
(e) Number of entries in closed arm

Radial arm maze:
Habituation session

A rat was placed in the central octagonal platform of the Radial arm maze placed on a wooden platform in a brightly lit room and allowed to move freely in the connected arms (Wooden, length 18.5 × height 3.5 × width 10.5 in) in which chocolate chips were spread about. The rats were removed from the maze after visiting all eight arms (one trial). After each trial the RAM was cleaned with alcohol to remove trace odours of the rewards. Habituation was carried out in a schedule of three trials/day for 5 days, with each trial separated by 2 h. During habituation, the rats were food-deprived to ≈ 30% of their normal daily dietary intake.

Training session

After habituation, the rats were trained in a schedule of three trials/day for 15 days. In these sessions, only 4 arms were selected as baited arm with chocolate and the task was replaced to make the correct arm choice to get the food reinforcement (reward). The rat was made to learn and memorize the particular arms in order to obtain the reward. In the first few trials, the food was stored simply at the proximal part of each selected arm. Then the food was moved to the middle of the arm for the next few trials. At the later trials (after 7 days of training), the food used to be placed at the distal end of the arm so the rat memorized the arm well.

Test trails

Following training, the rats were tested in each of the next 5 days (three trails/day, with 2h between trails). This established baseline values of memory for each rat

prior to any treatment. Each trail consisted of maximum 5 min duration and ended earlier if/when a rat had visited (and consumed reward in) all 4 baited arms. The same 4 arms were baited for each trial. All the trails were videotaped and coded. Two observers then independently evaluated the following parameters:

Number of re-entries in baited arms: if a rat re-entered a baited arm, it was considered as an error. The total number of re-entries in the baited arms (considered an indicator of working memory error (Mizuno et al., 2000)) for a rat in a trial (5 min duration or earlier as mentioned above) was reco rded; and Number of entries in non-baited arms: if a rat entered a non-baited arm, it used to be considered an error (i.e. indicator of reference reminiscence error). The total number of entries in all non-baited arms for one rat in a trial (5 min duration or earlier as mentioned above) used to be recorded.

These memory parameters have been again tested on all rats at post-treatment days (Sil S, Ghosh R 2016) 0, 7, 14, 21 and 28.

Parameters to be measured in Radial arm maze test are:

(a)Initial latency
(b)Time spent in non-baited arm
(c) Time spent in baited arm
(d) Number of entries in non-baited arm
(e) Number of entries in baited arm

Biochemical estimation

After completion of 28 days and all behavioral assessments animals were scarified by using cervical decapitation prior to deep anaesthesia. The brain removed and washed in ice cooled 0.9% normal saline kept on ice and subsequently blotted on filter paper then weighed and homogenized in bloodless phosphate buffer (0.1M pH 7.4). The homogenate were been centrifuged at 10000 rpm for 10 min at 4℃ (MPW- 350R, Korea) and post- mitochondrial supernatant (PMS) used to be used for the estimation of total protein, lipid peroxidation (LPO), Acetylcholinesterase (AChE), Tumor necrosis factor-alpha (TNF-alpha) and Interleukin-6 (IL-6). The fresh homogenate were centrifuged at 15000 rpm for 60 min at 4℃. The supernatant was once used for further estimation of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and total thiols (Anil K, Samrita D2009) and (AnilK, NehaS2006).

TNF-alpha to determine brain tissue levels of soluble TNFα, brain tissue was removed and homogenized in buffer containing 320 mmol/L sucrose, 1 mmol/L DL-dithiothreitol, 10 µg/mL leupeptin, 10 µg/mL soybean trypsin inhibitor 2µg/mL aprotinin, .2% Nonidet P40 and 50 mmol/L Tris brought to pH 7.0 at 20°C with HCl. The homogenates were centrifuged (13,000g for 10 min at 4°C) and supernatants were used for determinations with a commercially available rat enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (García-Bueno B, Madrigal JL2005).

IL-6 brain tissue were homogenized (7 v/w ratio) for 1 minute in a buffer of 10 mM HEPES-KOH, pH 7.9 (1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT, 1.0 mM AEBSF, 1g/mL leupeptin, 1.25g/mL pepstatin, and 0.1% Igeral). Homogenate were centrifuged at 12,000 g for 10 minutes at 4℃. The resulting supernatant was removed and IL-6 levels assessed in duplicate, 50 lL aliquots, using a specific ELISA (R&D Systems), according to the manufacturer's instructions. The protein level were determined by the Bradford (1976) method using reagents from Bio-Rad (Hercules, CA). Aliquots for total protein concentration was analysed using spectrophotometric assay at 540 nm in mg/mL based on a standard curve established for bovine serum albumin(Chatzipanteli K, Vitarbo E, Alonso OF 2012) Thiobarbituric acid reactive substances (TBARS) in the homogenate had been estimated by the method (Prabhakar et al 2006).Briefly, the 0.5 ml of 10% homogenate was once incubated with 15% TCA, 0.375% TBA and 5N HCL at 90-100℃ for 15 min, the mixture was once cooled and centrifuged at 2000 rpm for 10 min and absorbance of the supernatant measured at 532 nm against appropriate blank. The quantity of lipid peroxidation was once determined by the use of ε=1.56 X 105M-1cm-1 and expressed as TBARS/mg of protein(Mark JB, Robin LC, Jeffery FP 1987).

Superoxide dismutase activity were determined based totally on the capability of SOD to inhibit the auto-

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oxidation of epinephrine to adrenochrome at alkaline pH and had been estimated by way of the method (Misra and Fridovich, 1972) and (Ellman G, Diane CK, Valentino A1961).

Briefly, 25µl of the supernatant obtained from the brain added to a mixture 0.1 mM epinephrine in carbonate buffer (pH 10.2) in a total quantity of 1 ml and formation of adrenochrome was once measured at 295 nm. The SOD activity (U/mg of protein) used to be calculated by using the standard plot (HaraPM, Irwin F1972).

Catalase activity were measured by using the method of Claiborne (1985)13. Briefly the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1 ml hydrogen peroxide (0.019 M) and 0.05 ml tissue homogenate (10% w/v) in a total quantity of 3.0 ml. Changes in absorbance have been recorded at 240nm. Catalase activity used to be calculated in terms of nM H2O2 consumed/min/mg protein (KhynriamD, PrasadSB2003).

GSH estimated in various tissues by the method of Sedlak and Lindsay (1968). Briefly, 5% tissue homogenate have been prepared in 20 mM EDTA, pH 4.7100µl of the homogenate or pure GSH was once added to 0.2 M Tris-EDTA buffer (1.0 ml, pH 8.2) and 20mM EDTA, pH 4.7 (0.9 ml) followed by 20µl of Ellman's reagent (10mmol/l DTNB in methanol). After 30 min of incubation at room temperature, samples had been centrifuged and absorbance measured at 412 nm. (KhynriamD, Prasad SB2003).

Total thiols assay is based on the principle of formation of relatively stable yellow colour by using sulfhydryl groups with DTNB. (Briefly, 0.2 ml of brain homogenate was once mixed with 0.6 ml of phosphate buffer (pH 8.0), 40µl of 10 mM DTNB and 3.16 ml of methanol. This mixture was once incubated for 10 min at room temperature and centrifuge at 3000 rpm for 10 min (if required) and absorbance was once measured at 412 nm against appropriate blank. The total thiol content material was once calculated by the use of Ɛ= 13.6 x 103 M-1cm-1 as per) (Sedlak and Lindsay1968).

The protein concentration in samples was determined by the method of Lowry et al (1951). (Briefly, 0.5 ml of brain homogenate was mixed with 4.5 ml of 0.5 M NaoH and1 ml of this mixture was added to 10% of 1 ml TCA and centrifuge this mixture at 4000rpm for 10 min at 4^0C supernatant was discarded and ppt is dissolved in 1 ml of 0.5 M NaOH and freshly prepared 4ml of Alkaline mixture was added (2gmofNa2CO3,100ml of 0.1 NaOH, 1 ml of 4% CuSO4) Wait for 10 min (keep in dark place) add 0.5 ml of follen's reagent wait for 10min(keep in dark place) absorbance was recorded at 540nm).

The method of AChE activity estimation is popularly known as Ellman's method named after George Ellman's who developed this method in 1961. The esterase activity was measured by providing an artificial substrate acetylthiocholine(ATC).Thiocholine released because of the cleavage of ATC by Ach Eis allowed to react with–SHreagent5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) which is reduced to thio nitrobenzoic acid a yellow-colored anion with an absorption maxim at 412nm.The extinction coefficient of the thionitrobenzoic acid is$1.36x10^4$/molar/centimeter. The concentration of thionitrobenzoic acid detected using a UV spectrophotometer is then taken as a direct estimate of the AChE activity.

Histopathological studies

Histopathological studies suggested that the control group showed marked intracellular space increased and more vacuoles seen, density of cells decreased, architecture completely altered, also haemorrahage and neuronal cell death was observed in cerebrum. A significant protection was provided by ethanol extract of *Phoenix sylvestris* (EPS) treated groups by reducing infiltration of neutrophils, intracellular space, architecture was regained to normal and decreased necrosis was seen in EPS treated 150mg/kg, 300mg/kg and 500mg/kg, groups. This significant recovery EPS treated groups was comparable with sham group.

Statistical Analysis

All the values are expressed as mean±SEM, n=6, [One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test] maximum significant $^a p<0.001$, as compared to sham group and minimum significant $*p<0.05$,$**p<0.01$&$***p<0.001$maximum significant

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valve as compared to control group.

Results

Molecular Docking Studies

The biological activity of *Phoenix sylvestris* compounds towards the TNF-alpha converting enzyme (PDB ID: 2 fV5) and Acetylcholinesterase (PBD ID: 4EY5) have been evaluated *in-silico*, the 3D structure of the receptor retrieved from protein data bank site of TNF-alpha converting enzyme (PDB ID: 2fV5) and Acetylcholinesterase (PBD ID: 4EY5). For the selected compounds and protein, the docked binding mode used to be established to link the docking score function. The binding pattern analysis between TNF-alpha converting enzyme (PDB ID: 2 fV5), Acetylcholinesterase (PBD ID: 4EY5) and ligands suggested that the binding pattern varied with the ligant nature. The docking results of the *Phoenix sylvestris* phytocompounds are given in Fig-1. Docking studies show that the ligands bind to the active site region of TNF-alpha converting enzyme (PDB ID: 2fV5) and Acetylcholinesterase (PBD ID: 4EY5). In the docking results had been represented in the form of e-negative values. If greater the negative e-values represent high binding affinity between the receptor and ligand molecules, indicating the greater efficiency of the bioactive compounds.

Fig.1: Molecular Docking studies: 2D & 3D interaction of phytocompounds ofPhoenix sylvestries with TNF-α converting enzyme (TACE) PDB ID: 2FV5 crystal structure using Auto Dock.

Diethyl nitrosamine

2,4-Di-tert-butylphenol

2, 3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one

4-methylcatechol

Diethyl Phthalate

Molecular Docking studies: 2D& 3Dinteraction of phytocompounds of*Phoenix sylvestries* with TNF-α converting enzyme (TACE) PDB ID: 2FV5 crystal structure using Auto Dock.

Diethyl nitrosamine

2, 3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one

4-methylcatechol

2,4-Di-tert-butylphenol

Diethyl Phthalate

In docking studies, the phytochemical compound from *Phoenix sylvestris* like 2, 3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-oneshowed the binding affinity with -7.1 kcal/mol towards TNF-α converting enzyme and formed 4 conventional hydrogen bonds, 1 alkyl bond and 1 pi-alkyl bond with amino acid residues

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TYR(B:433), VAL(B:434), SER(B:441), VAL(B:440), LEU(B:401) and HIS(B:405) respectively. The phytochemical compound of *Phoenix sylvestris* like4-methylcatechol showed the binding affinity with -6.8 kcal/mol towards TNF-α converting enzyme and formed 2 conventional hydrogen bonds, 2 alkyl bonds and1 pi-alkyl bonds with amino acid residues TYR(B:433), VAL(B:440), VAL(B:402), LEU(B:402) and ALA(B:439) respectively. The phytochemical compound of *Phoenix sylvestris* like 2,4-Di-tert-butylphenol demonstrated the binding affinity with -6.1 kcal/mol towards TNF-α converting enzyme and formed 2 conventional hydrogen bonds, 1 alkyl bond, 1 pi-alkyl bond, 1 pi-donor hydrogen bond and 1 pi-sigma bond with amino acid residues ALA(B:439), LEU(B:348), VAL(B:402), GLY(B:349), GLU(B:406) and HIS(B:405) respectively. The phytochemical compound of *Phoenix sylvestris* like Diethyl Phthalate showed the binding affinity with -5.9 kcal/mol towards TNF-α converting enzyme and formed 3 conventional hydrogen bonds and 1 pi-alkyl bond with amino acid residues LEU(A:348), GLY(A:349), ALA(A:439) and VAL(A:402) respectively. The phytochemical compound of *Phoenix sylvestris* like Diethyl nitrosamine showed the binding affinity with -5.2 kcal/mol towards TNF-α converting enzyme and formed 1 conventional hydrogen bond, 1 C-H bond, 1 unfavourable donor-donor bond and 2 alkyl bonds with amino acid residues TYR(B:433), LEU(B:401), VAL(B:440), SER(B:441) and VAL(B:434) respectively.

The phytochemical compound *Phoenix sylvestris* like 2,4-Di-tert-butylphenol from showed the binding affinity with -7.9 kcal/mol towards Acetylcholinesterase enzyme and formed 1 pi-sigma bond and 1 pi-pi stacked bond with amino acid residues TRP(A:286) and TYR(A:341) respectively. The phytochemical compound of *Phoenix sylvestris* like Diethyl Phthalate demonstrated the binding affinity with -6.7 kcal/mol towards Acetylcholinesterase enzyme and formed 1 alkyl bond, 2 pi-alkyl bonds, 1 pi-pi stacked bond and 1 pi sigma bond with amino acid residues TRP(A:286), LEU(A:289), TYR(A:337), PHE(A:338) and TYR(A:341) respectively. The phytochemical compound of *Phoenix sylvestris* like showed the binding affinity with -6.0 kcal/mol towards Acetylcholinesterase enzyme and formed 2 conventional hydrogen bonds, 1 pi-pi stacked bond and 3 pi-alkyl bonds with amino acid residues SER(B:293), ARG(B:296), TRP(B:286), TYR(B:341), TYR(B:124) and PHE(A:297) respectively. The phytochemical compound of *Phoenix sylvestris* like 2, 3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-oneshowed the binding affinity with -5.9 kcal/mol towards Acetylcholinesterase enzyme and formed 2 conventional hydrogen bonds, 1 alkyl bond and 1 pi-alkyl bonds with amino acid residues ASN(A:533), ALA(A:412), TRP(B:385) and ALA(A:530) respectively. The phytochemical compound of *Phoenix sylvestris* like Diethyl nitrosamine showed the binding affinity with -4.8 kcal/mol towards Acetylcholinesterase enzyme and formed 1 conventional hydrogen bond with amino acid residues LYS(A:332).

a). Morris' water maze test

The result of this study showed that *Phoenix sylvestris* has a potential neuroprotective activity against colchicine induced neurobehavioral study of Neuroinflammation and cognitive deficits. The result of behavioral assessment of Morris water maze was summarized. The escape latency L1, L2, L3, L4 on the Days 7, 14, 21 and 28 showed the significant (p<0.001) with increased time latency to locate invisible platform in control group as compared to sham group. In contrast with ethanol extract of *Phoenix sylvestris* treatment groups on Day 7, 14, 21 and 28 showed significant (p<0.01 to p<0.001) result with decreased at different doses of EPS 150mg/kg, 300mg/kg & 500mg/kg shows significant (p<0.001) in escape latency L1 and at a dose of 150mg/kg, 300mg/kg & 500mg/kg shows significant (p<0.001) in escape latency L2and at a dose of 150mg/kg, 300mg/kg & 500mg/kg shows significant (p<0.01) in escape latency L3 and at a dose of 150mg/kg, 300mg/kg & 500mg/kg shows significant (p<0.01) in escape latency L4. On Day 14 at a dose of 150mg/kg, 300mg/kg & 500mg/kg showed significant (p<0.001) in latency L1, L2, L4 and at a dose of 150mg/kg, 300mg/kg, and 500mg/kg showed significant (p<0.01) in latency L3. On Day 21 at a dose 150mg/kg, 300mg/kg, 500mg/kg showed significant (p<0.001). On Day 28 after the dose of 150mg/kg, 300mg/kg, and500mg/kg showed significant (p<0.001) in latency L1, L2, L3 and L4 showed reduction in time of escape latency to locate invisible platform as compared with control group.

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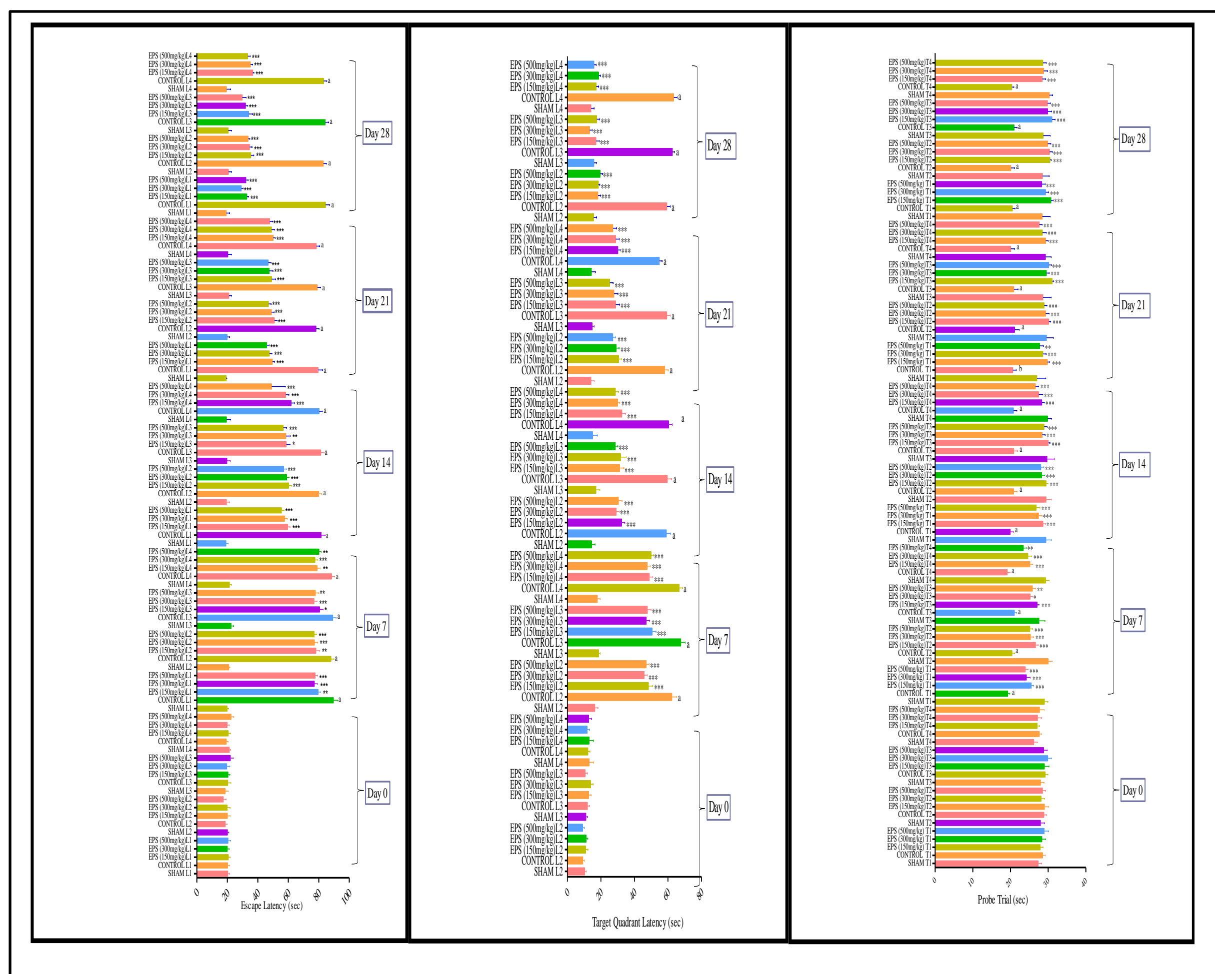
Effect of ethanol extract of *Phoenix sylvestris* (EPS) shows significant result in time spent in platform quadrant. Days 7, 14, 21 and 28 the target quadrant latency L2, L3, L4 showed the significant (p<0.001) result with decreased the time spent in the target quadrant, in control group as compared to sham group whereas in contract with EPS treated groups at a different dose of 150mg/kg, 300mg/kg, and500mg/kg showed significant increase in time spent in the target quadrant latencies as compared to control group.

The Probe trail is a significant difference in special bias of animals towards the target quadrant of the pool that contained the invisible platform during the escape latency. The result of Probe trail Similarly the Days 7, 14, 21 and 28 the Probe trail T1, T2, T3 showed the significant (p<0.001) result with decreased the time spent in the target quadrant, when control group as compared to sham group. In contrast with ethanol extract of *Phoenix sylvestris* treated groups on Day 7, 14, 21 & 28 at the dose 150mg/kg, 300mg/kg & 500mg/kg showed significant increase (p<0.001) in probe trail T1, T2, T3 & T4 and at a dose of 150mg/kg, 300mg/kg & 500mg/kg as compare to control group.

Fig.2: Effect of ethanol extract of Phoenix sylvestrieson Escape latency, Target quadrant latency and Probe trail of Morris water maze test in Colchicine induced Neuroinflammation and cognitive deficits.

(b).



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Elevated Plus Maze Test

The effect of ethanol extract of *Phoenix sylvestris* shows significant resulted in initial transfer latency of elevated plus maze test summarized in Table 1. Ininitial transfer latency. Retention transfer latency (enter into closed arm) (2nd RTL and 3rd RTL) showed the stable as compared to ITL on Day 0 in sham group. In contrast the control group rats showed significant (p<0.001) increase in RTL on Days 7, 14, 21 and 28 as compared to Day 0 and sham group where as In EPS treated rats on Day 7, 14, 21 & 28 at a dose of 150mg/kg, 300mg/kg & 500mg/kg shows a significant (p<0.001) in reduced the transfer latency as compared with control group. Similarly, on 14, 21, and 28Days dose of 150mg/kg, 300mg/kg & 500mg/kg shows a significant (p<0.001) in shorter the transfer latency as compared with control group.

The effects of EPS on colchicine induced Neuroinflammation and cognitive deficits in the elevated plus are summarized in (Table1). In elevated plus maze test control group shows significant (p<0.001) increase in time spent in open arm, and number of entries in open arm as compared to sham group on the 7, 14, 21 and 28 Days. On EPS treatment at a dose of 150mg/kg, 300mg/kg & 500mg/kg shows a significant (p<0.001) resulted in decrease in time spent in open arm, and number of entries in open arm and on 21 and 28 Day as compared with control group.

Similarly, In elevated plus maze test control group shows significant (p<0.001) decrease in time spent in closed arm as compared to sham group and significant (p<0.05, p<0.001, and p<0.01) decrease in number of entries in closed arm as compared to sham group onthe7,14, 21 and28 Days. On EPS treatment at a dose of 150mg/kg, 300mg/kg and 500mg/kg shows a significant (p<0.001) result in increase in time spent in closed arm and number of entries in closed arm on 21 and 28 Day as compared with control group.

Table.1: Effect of ethanol extract of *Phoenix sylvestries* on initial transfer latency, time spent in open arm, time spent in closed arm, number of entries in open arm and number of entries in closed arm of plus maze test in Colchicine induced Neuroinflammation and cognitive deficits.

Treatment Groups	Initial transfer latency(in sec)					Time spentin Open arm(in sec)					Time spent in Closed arm(in sec)				
	Day-0	Day- 7	Day-14	Day-21	Day-28	Day-0	Day- 7	Day-14	Day-21	Day-28	Day-0	Day-7	Day-14	Day-21	Day-28
Sham	14.1±1.82	19.1±3.48	18.3±3.10	20.±2.0	24.8±2.57	62.3±6.31	64.1±8.30	57.6±7.03	59.3±6.71	56.8±5.70	237.7±6.31	235.8±8.30	242.3±7.03	240.7±6.71	243.2±5.70
Control	16.3±1.43	73.0±2.07^{a}	69.1±2.48^{a}	68.±2.12^{a}	66.6±2.29^{a}	57.0±5.72	229.2±7.68^{a}	198.8±7.50^{a}	196.5±6.9^{a}	200±13.1^{a}	243.0±5.72	70.83±7.687^{a}	101.2±7.50^{a}	103.5±6.9^{a}	99.8±13.1^{a}
EPS (150mg/kg)	16.0±2.06	61.1±1.98**	59.3±2.57*	49.9±2.1***	39.5±1.60***	58.5±5.70	206.0±7.66	145.0±6.77***	77.67±9.47***	39.6±5.11***	241.5±5.70	94.00±7.66	155.0±6.77***	222.3±9.47***	260.5±5.07***
EPS (300mg/kg)	17.1±1.24	49.2±2.26***	45.0±1.61***	40.0±2.3***	36.2±1.65***	51.0±4.13	225.7±5.09	67.00±6.69***	60.67±7.89***	30.5±3.42***	249.0±4.13	74.33±5.090	233.0±6.69***	239.3±7.89***	269.5±3.42***
EPS (500mg/kg)	15.7±2.04	41.8±1.65***	40.1±1.95***	38.4±1.53***	34.8±1.62***	54.0±9.00	217.8±5.31	54.17±5.70***	32.5±3.871***	27.3±4.23***	246.0±9.00	82.17±5.31	245.8±5.70***	267.5±3.87***	272.7±4.23***

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Treatment Groups	Number of entries in Open arm (in sec)					Number of entries in Closed arm (in sec)				
	Day-0	Day-7	Day- 14	Day-21	Day- 28	Day-0	Day- 7	Day- 14	Day- 21	Day- 28
Sham	5.66±0.494	3.3±0.33	3.0±0.36	3.1±0.30	3.3±0.33	3.8±0.30	3.6±0.33	4.5±0.50	5.0±0.36	5.0±0.36
Control	7.3±0.55 [c]	8.0±0.25 [a]	7.5±0.42 [a]	8.5±0.42 [a]	9.3±0.42 [a]	3.6±0.33	3.1±0.30	3.1±0.3 [c]	3.8±0.30 [c]	3.8±0.3 [c]
EPS (150mg/kg)	5.8±0.30	6.0±0.36*	3.5±0.42***	5.6±0.42***	7.3±0.33**	3.5±0.42	2.6±0.33	6.0±0.36***	7.16±0.30***	7.1±0.30***
EPS (300mg/kg)	3.8±0.43*	5.6±0.33**	2.8±0.30***	4.0±0.36***	6.0±0.36***	3.1±0.30	3.3±0.33	6.5±0.22***	8.5±0.22***	8.5±0.22***
EPS (500mg/kg)	4.1±0.30	5.5±0.84**	2.8±0.30***	3.8±0.30***	5.8±0.30***	4.1±0.40	3.8±0.30	7.1±0.30***	9.16±0.30***	9.1±0.30***

All the values are expressed as mean±SEM, n=6,[One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test] $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ as compared to sham group and $*p<0.05$, $**p<0.01$, $***p<0.001$ as compared to control group.

c). Radial arm maze

The effect of ethanol extract of *Phoenix sylvestris* shows significant results in initial transfer latency of radial arm maze test summarized. The control group rats showed significant ($p<0.001$) increase in RTL on Days 7, 14, 21, and 28 as compared to Day 0 and sham group. In EPS treated rats on Day 7 at a dose of 150mg/kg, 300mg/kg & 500mg/kg shows a significant ($p<0.001$) in reduced the transfer latency as compared with control group. Similarly, on 14, 21 and 28Days dose of 150mg/kg, 300mg/kg & 500mg/kg shows a significant ($p<0.001$) in shorter the transfer latency as compared with control group Table 2.

The effects of EPS on colchicine induced Neuroinflammation and cognitive deficits in the radial arm maze are summarized. In radial arm maze test on 7, 14, 21 and 28 Days control group shows significant ($p<0.001$) increase in time spent in non-baited arm, and number of entries in non-baited as compared to sham group. On EPS treatment at a dose of 150mg/kg, 300mg/kg & 500mg/kg shows a significant ($p<0.001$) resulted in decrease in time spent in non-baited arm and number of entries in non-baited arm on 21 and 28 Day as compared with control group.

Similarly, In radial arm maze test control group shows significant ($p<0.001$) decrease in time spent in baited arm as compared to sham group and significant ($p<0.05$ to$p<0.001$) decrease in number of entries in baited arm as compared to sham group onthe7, 14, 21 and28 Days. On EPS treatment at a dose of 150mg/kg, 300mg/kg& 500mg/kg shows a significant ($p<0.001$) result in increase in time spent in baited arm and number of entries in baited arm on 21 and28 Day as compared with control group.

Table.2: Effect of ethanol extract of *Phoenix sylvestries* (EPS) on latency, time spent in non-baited arm, time spent in baited arm, number of entries in non-baited arm and number of entries in baited arm of plus maze test in Colchicine induced Neuroinflammation and cognitive deficits.

Treatment Groups	Latency (in sec)					Time spent in non-baited arm(in sec)					Time spent in baited arm(in sec)				
	Day-0	Day- 7	Day-14	Day-21	Day-28	Day-0	Day- 7	Day-14	Day-21	Day-28	Day-0	Day-7	Day-14	Day-21	Day-28
Sham	13.4±1.71	13.5±1.61	13.7±1.38	13.1±1.3	13.0±1.21	63.0±6.85	59.2±7.56	60.5±6.92	62.3±6.71	3.33±0.21	3.83±0.30	242.7±	4.66±0.33	237.7±	4.8±0.47

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												6.37		6.71	
Control	13.7±1.77	39.2±1.54^{a}	38.1±1.63^{a}	38.03±1.7^{a}	38.0±1.54^{a}	55.5±7.25	227.2±5.400^{a}	201.8±7.50^{a}	199.5±6.9^{a}	9.33±0.49^{a}	3.83±0.30	74.50±5.48^{a}	3.3±0.33^{c}	110.5±5.9^{a}	4.8±0.30^{a}
EPS (150mg/kg)	14.2±2.07	28.9±2.09***	27.7±2.25***	25.9±2.5***	22.9±2.17***	57.6±6.20	207.2±7.129	148.0±6.77***	80.6±9.47***	6.33±0.33***	3.50±0.42	91.1±8.01	6.3±0.42***	237.7±13.5***	8.1±0.30***
EPS (300mg/kg)	15.5±1.93	24.50±1.60***	23.5±1.46***	21.4±1.6***	18.6±1.58***	57.0±4.43	228.2±5.016	70.1±6.58***	63.6±7.89***	5.0±0.36***	3.50±0.42	71.8±5.01	7.5±0.22***	238.0±8.18***	9.5±0.42***
EPS (500mg/kg)	13.81±2.30	19.8±1.41***	18.8±1.41***	16.8±1.5***	13.8±1.49***	53.3±6.88	218.7±5.402	57.1±5.70***	35.5±3.87***	4.83±0.30***	3.66±0.42	80.8±5.52	7.83±0.40***	264.5±3.87***	10.3±0.66***

Treatment Groups	Number of entries in non-baited arm (in sec)					Number of entries in baited arm (in sec)				
	Day-0	Day- 7	Day- 14	Day- 21	Day- 28	Day-0	Day- 7	Day- 14	Day- 21	Day- 28
Sham	5.3±0.61	4.0±0.36	3.6±0.33	3.66±0.55	3.3±0.21	3.8±0.30	4.3±0.49	4.6±0.33	5.1±0.30	4.8±0.47
Control	6.0±0.36	8.0±0.36^{a}	8.16±0.30^{a}	8.5±0.42^{a}	9.3±0.49^{a}	3.8±0.30	3.3±0.33	3.3±0.33^{c}	3.3±0.33^{c}	4.8±0.33^{a}
EPS (150mg/kg)	5.5±0.42	6.8±0.47	4.16±0.47***	4.6±0.42***	6.3±0.33***	3.5±0.42	3.0±0.44	6.3±0.42***	7.1±0.30***	8.1±0.30***
EPS (300mg/kg)	5.3±0.71	5.8±0.47*	2.83±0.30***	3.5±0.42***	5.0±0.36***	3.5±0.42	3.8±0.60	7.5±0.22***	8.5±0.22***	9.5±0.42***
EPS (500mg/kg)	5.5±0.56	5.0±0.77**	2.50±0.22***	3.0±0.36***	4.8±0.30***	3.6±0.42	4.6±0.88	7.8±0.40***	0.1±0.30***	10.3±0.66***

All the values are expressed as mean±SEM, n=6, [One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test] $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ as compared to sham group and $*p<0.05$, $**p<0.01$, $***p<0.001$ as compared to control group.

Effects of ethanol extract of on *Phoenix sylvestris* (EPS) inflammatory mediators

The inflammatory markers were summarized in Table 3.

The brain homogenate from the control group showed significant increase in TNF-α ($p<0.001$) and significant decreased in IL-6 ($p<0.001$) as compared to sham group where as in treatment group shows significant.

Table.3. Effect of ethanol extract of *Phoenix sylvestris* (EPS on inflammatory mediators and biochemical estimation in Colchicine induced Neuroinflammation and Cognitive deficits.

Treatment groups	TNF-α	IL-6	Lipid peroxidation (nmoles/mg of protein)	GSH (nmoles/mg of protein)	Total thiols (µmoles/mg of protein)	SOD (U/mg of protein)	Catalase(U/mg of protein)
Sham	50.05±2.11	5.978±0.24	313.1±25.1	34.00±2.61	108.7±2.89	134.9±9.37	0.048±0.002
Control	113.4±3.91^{a}	2.80±0.06^{a}	500.5±11.3^{a}	13.9±0.80^{a}	33.2±1.52^{a}	86.5±4.19^{b}	0.03±0.0007^{a}
EPS (150mg/kg)	95.01±1.43***	4.88±0.098***	394.5±28.5**	34.82±1.25***	42.9±1.28**	111.6±3.01	0.044±0.0012**

EPS (300mg/kg)	81.89±2.18***	5.77±0.13***	298.7±11.5***	48.75±1.76***	84.03±0.63***	129.6±11.3**	0.060±0.0029***
EPS (500mg/kg)	67.6±1.77***	5.6±0.17***	210.4±19.1***	63.57±2.87***	108.0±2.05***	138.9±11.1**	0.081±0.0025***

All the values are expressed as mean±SEM, n=6, [One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test][a]$p<0.001$, [b]$p<0.01$, [c]$p<0.05$ as compared to sham group and *$p<0.05$,**$p<0.01$, ***$p<0.001$ as compared to control group.

Effects of ethanol extract of on *Phoenix sylvestris* (EPS) biochemical estimations

The biochemical estimations were summarized in Table 4. The brain homogenate from the control group showed significant increase in LPO ($p<0.001$) and significant decreased in GSH ($p<0.001$), Total thiols ($p<0.001$), SOD ($p<0.01$) & Catalase ($p<0.001$) as compared to sham group. In contrast with EPS treated groups 150mg/kg, 300mg/kg and 500mg/kg shows significant protection by reducing elevated level of LPO ($p<0.01$ &$p<0.001$) and shows significant increased level of non-enzymatic antioxidant like GSH ($p<0.001$) and total thiols ($p<0.01$ &$p<0.001$) increased levels of antioxidants enzymes the SOD ($p<0.01$) Catalase ($p<0.01$ &$p<0.001$) as compared with control group.

Effect of ethanol extract of *Phoenix sylvestris* (EPS) on Acetylcholinesterase (AChE) Activity

The effect of Acetylcholinesterase (AChE) Activity on i.c.v administrated colchicines induced Neuroinflammation and cognitive deficits was summarized in Table 4.The brain homogenate from the control group showed significant ($p<0.001$) increased AChE level at 2min, 4min, 6min, 8min and 10min as compared to sham group. In contrast EPS treated group at a dose of 150mg/kg, 300mg/kg & 500mg/kg showed a significant ($p<0.001$) decrease of AChE at 2min, 4min, 6min, 8min and10min as compared with control group Table 4.

Table.4. Effect of ethanol extract of *Phoenix sylvestris* (EPS) on Acetylcholinesterase activity in Colchicine induced Neuroinflammation and Cognitive deficits.

Treatment groups	Acetylcholinesterase activity					
	0 min	2 min	4 min	6 min	8 min	10 min
Sham	2.270±0.0875	5.770±0.1874	3.780±0.5448	6.608±0.4854	10.53±0.4813	15.97±0.6944
Control	3.620±0.1636[a]	6.585±0.1898	8.308±0.273 [a]	10.28±0.4412[b]	15.82±0.7000[a]	19.29±0.5898[c]
EPS (150mg/kg)	.368±0.1015***	5.288±0.3893*	5.638±0.8146**	7.095±0.9963**	13.39±0.4844*	16.25±0.9728*
EPS (300mg/kg)	1.598±0.1517***	3.968±0.2551***	4.865±0.4168***	5.833±0.5409***	11.52±0.5106***	13.53±0.8505***
EPS (500mg/kg)	1.225±0.0988***	3.243±0.3811***	3.715±0.2950***	4.830±0.4311***	10.36±0.3787***	12.22±0.5120***

All the values are expressed as mean±SEM, n=6, [One way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test] [a]$p<0.001$, [b]$p<0.01$, [c]$p<0.05$ as compared to sham group and *$p<0.05$, **$p<0.01$, ***$p<0.001$ as compared to control group.

Effect of ethanol extract of *Phoenix sylvestris* (EPS) on Histopathological studies

Histopathological studies in Fig.3 suggested that the control group showed marked intracellular space and more vacuoles seen, density of cells decreased, architecture completely altered, also haemorrahage and neuronal cell death were observed in cerebrum. A significant protection was provided by ethanol extract of *Phoenix sylvestris* (EPS) treated groups by reducing infiltration of neutrophils, intracellular space,

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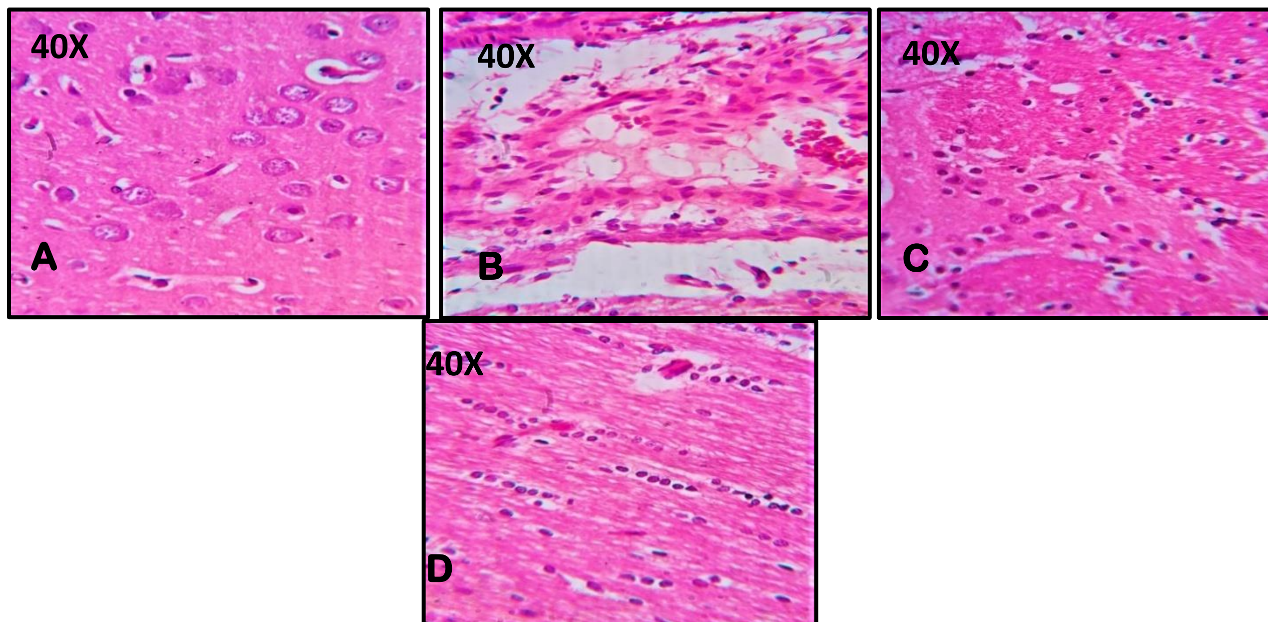
architecture was regained to normal and decreased necrosis was seen in EPS treated 150mg/kg, 300mg/kg and 500mg/kg groups. This significant recovery on EPS treated groups was compared with sham group Fig 3.

Fig.3. Effect of ethanol extract of Phoenix sylvestris (EPS) in Colchicine Neuroinflammation and cognitive deficits in rat.



Photograph of brain section of different groups stained with Haemotoxylin and Eosin. Images; A: Sham group, B: Control group, C:EPS (150mg/kg), D: EPS(300mg/kg), E: EPS (500mg/kg); In Images B there was marked decrease in density of cells, intracellular space is increased, architecture completely damaged, increased neuronal cell death was observed. But in EPS treated group (C, D & E), cell density was increased, intracellular space and cell vacuoles were decreased, normal architecture was regained, reduced neuronal cell death observed. This protection in treatment groups was mostly comparable with shamgroup (A).EPS =Ethanol extract of *Phoenixsylvestris.*

Discussion

The brain is an important organ that controls the features of all the different organs in the human body through neuronal connectivity and neuronal signal transmission. Central nervous system (CNS) is the most complex and very poorly understood structure in the human body. The neuronal circuits in the CNS and its functions are impaired in neuroinflammatory diseases (Busche MA, Konnerth A2016). Inflammation is a shielding mechanism in the body that features to repair, regenerate and remove the damaged tissues/cells or infective agents, parasites or toxins from the body (Kulkarni OP, Lichtnekert J2016).

Inflammatory responses are carried out by way of several immune and inflammatory cells which include T-cells, neutrophils, macrophages, microglia and mast cells. Similarly, neuroinflammation is a shielding mechanism to restore the damaged glial cells and neuronal cells in the CNS. Neuroinflammation is mediated by means of microglia-the resident brain macrophage, astrocytes, neurons, T-cells, neutrophils, mast cells and inflammatory mediators released from these cells(Shabab T, Khanabdali R2016).Contrary to different cells, as soon as the neurons are damaged or degenerated, they are unable to be repaired or regenerate in the CNS (Ransohoff RM2016).

Neuroinflammation is at the beginning a shielding response in the brain, but excess inflammatory responses are detrimental and in fact, it inhibits the neuronal regeneration. Chronic neuroinflammation plays an important role in the onset and development of

neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and Multiple Sclerosis (MS) (Russo MV, McGavern DB2016). Neuroinflammation factors such as normal ageing process, dementia, trauma, stroke, hypertension, depression, diabetes, tumors, infections, toxins and drugs can initiate neuroinflammation in the CNS (Chen WW, Zhang X, Huang WJ2016).

Normal aging process reason decreased neurogenesis, increased synaptic aberrations, greater metabolic stress, augmented neuroinflammation, cognitive decline, neurobehavioral deficits and increased reactivity to any immune challenges. Moreover, aging is also associated with increased systemic inflammation, increased BBB permeability, impaired glial cell signaling and chronic mild pro inflammatory reactions in the CNS cells (Barrientos RM, Kitt MM2015).

Microglia in the aged brain show dystrophic morphology (inflammatory), elevated expression of inflammatory markers and decreased expression of neuroprotective factors(Von Bernhardi R, Eugenin-von Bernhardi L2015).Microglial cells mediate communication between the CNS and immune cells and this conversation is impaired in the aged predisposing to low-grade and chronic inflammation and the onset of neurodegenerative diseases(Niraula A, Sheridan JF2016).

Low-grade chronic neuro-inflammation associated with normal aging contributes to cognitive deficits and increased susceptibility to different age-related pathologies. A higher understanding of the mechanisms responsible for neuro-immune dysfunctions related with older age is important for better therapy options (Zilka N, Kazmerova Z, Jadhav S2012). Further, aging process induces various cell changes such as increased intracellular Ca2+ ranges that can lead to low-grade inflammation in the CNS and in the peripheral systems (Matt SM, Johnson RW2016).

This low-grade inflammation induces the release of inflammatory mediators including interleukin-1beta (IL-1β), IL-6 and tumor necrosis factor-alpha (TNF-α). Astrocytes and microglia characteristic as inflammatory cells and release many neuroinflammatory cytokines and chemokines inside the aging CNS (Ward RJ, Dexter DT2015). This chronic increase of inflammatory mediator ranges in the aged human beings make them extra prone to neuroinflammation in the CNS mainly to the onset of neurodegenerative diseases(Bruuns gaard H, Pedersen M2001).

Neuroinflammation due to inflammatory mediator overexpression is related with behavioral disturbances; increased IL-1 expression in Alzheimer brain is directly associated to plaque formation, progression and neuronal overexpression of acetylcholinesterase. The TNF-α, IL-1β and IL-6 overexpression stimulated production of amyloid-β which is crucial for neuro-degeneration(Iwalewa EO, McGaw LJ, Naidoo V2007).The selected ligands (*Phoenix sylvestris* fruit extract) have been subjected to *in-silico* learn about to discover there binding mode with TNF-alpha converting enzyme (PDB ID:2fV5) and Acetylcholinesterase (PBD ID:4EY5) the using of Autodock tools 1.5.6. has been studied.

Tumor necrosis factor-alpha (TNF-α) –converting enzyme (TACE), a member of the family of metalloproteinase disintegrin proteins, is responsible for the conversion of inactive TNF-α precursor from to energetic mature form. TNF-α is a pleiotropic cytokine that contributes to cellular immunity and inflammatory response in large range of inflammatory pathologies. Although a large range of research indicate the use of TACE inhibitors, which prevents processing of TNF-α as potential therapeutic drugs for the therapy of inflammatory diseases (Grisaru D, Sternfeld M1999).

Acetylcholinesterase (AChE) is an enzyme and its role is to terminate cholinergic neurotransmission by means of hydrolysis of Acetylcholine. The percent strategy relies on the increase in the synaptic availability of acetylcholine to compensate the cholinergic deficit that arises from neuronal loss. Molecular docking, which is a significant technique to illustrate the affinity in the receptor-ligand complexes in the current study. The phytocompounds from the *Phoenix sylvestries* have been selected and subjected to insilico studies, to discover their binding mode with Acetylcholinesterase (Ganesh R, Kannan I 2017 and Ramana KV2010) (AChE) PDB ID:4EY5 the usage of Autodock tools 1.5.6.

Docking studies show that the ligands bind to the active site region of TNF-alpha converting enzyme (PDB ID: 2fV5) and Acetylcholinesterase (PBD ID: 4EY5)

enzyme with excellent binding energy. The docking results had been represented in the form of e-negative values. In the docking studies, greater negative e-values represent high binding affinity between the receptor and ligand molecules, indicating the greater efficiency of the phytocompounds. The docked ligands show scores ranging from -5.2 to -7.1 kcal/mol in TNF-alpha converting enzyme (PDB ID: 2fV5) and -4.8 to -7.9 kcal/mol in Acetylcholinesterase (PBD ID: 4EY5). The obtained results suggested that binding energy of three phytocompounds are lower than - 6ckal/mol, indicating that they should fit well into the binding pocket of the enzyme and predicted neuroinflammatory activity.

The current study confirmed that effect of ethanol extract of *Phoenix sylvestris* in the prevention of Neuroinflammation and cognitive deficits i.c.v administration colchicine induced rats. The present study showed the treatment with EPS improved impaired cognition, reduced the malondialdehyde (MDA) level and restored the lowered GSH, increased glutathione-s-transferase, total thiols, Catalase and SOD. This study suggests that i.c.v administration of colchicine is characterized by step by step deterioration of learning and memory, oxidative stress, and decrease in acetylcholine level (Bensimon G, ChermatR 1991) and (Nakagawa YS, Nakamura S1987).

In this study i.c.v administration of colchicine resulted in significant memory impairment were observed in Morris water maze, elevated plus maze and radial arm maze test of rats this memory impairments which used to be reduced by treatment with EPS. This study showed administration of EPS used to be capable of enhancing the Neuroinflammation and cognitive deficit effects. Lipid peroxidation plays an important role in oxidative damage of lipids. The essential metabolites of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxynonenal (HNE) (SayreLM,ZelaskoDA1997). This study additionally confirmed that the i.c.v administration of colchicine confirmed the increased levels of malondialdehyde (MDA) and are responsible for the oxidative damage in rats. Glutathione is an endogenous antioxidant in decreased form within the cells and react with and prevent the generation of hydroxyl free radicals (BainsJS,ShawCA1997). The GSH level and glutathione-S-transferase activity decreased in colchicine treated animals indicates there is decreased activity of glutathione system in inhibiting oxidative stress and increased generation of free radicals. Treatment with EPS was once able to restore the level of GSH and a significant increase in the glutathione-S-transferase activity.

Central administration of colchicine confirmed marked destruction of the hippocampal granule cells and septo-hippocampal pathways this resulted in loss of cholinergic neurons and decreased activities of acetylcholinesterase and choline acetyltransferase (McClureWO1975).

This study indicates that administration of colchicine significantly increased the acetylcholinesterase activity thereby leading to learning and memory deficits. EPS used to be able to reduce and improve the colchicine induced neurotoxicity and also decrease the AChE activity.

In conclusion, the present study suggests that administration of ethanol extract of *Phoenix sylvestris* (EPS) possesses neuroprotective activity against colchicine induced Neuroinflammation and cognitive deficits.

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