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Behavioral effect of phosphatidylcholine isolated from soy lecithin in streptozotocin induced experimental alzheimers model

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Abstract
Background: Phospholipids are lipids containing a phosphoric acid residue; they are nature's principal surface-active agents. They are found in all living cells. Neurodegenerative disorders are characterized by progressive irreversible loss of neuron from specific region of brain.
Objectives: The present research work was designed to investigate the effects of different fractions of Phosphatidylcholine (PC), its antioxidant property in streptozotocin induced Alzheimer's disease in mice.
Methods: Intracerebroventricular administration of Streptozotocin to induce cognitive dysfunction & oxidative stress as an Alzheimer's disease in Swiss albino mice. Morris water maze, Elevated plus Maze were used to study cognitive behavior.
Results: Treatment of ICV STZ produced a significant decrease in MWM and EPM performance of mice hence reflecting loss of learning and memory. The study of the memory in Alzheimer's disease was found that PC 95 and 55 (200 and 300mg/kg) treated STZ-injected mice showed significant results. The time spent with target quadrant in MWM and retention latency in EPM was increases with PC as compared to STZ group.
Conclusion: Phosphatidylcholine showed improvement in cognitive impairment & it is a good promising candidate for increasing memory in Alzheimer's disease. The study demonstrates the effectiveness of PC in preventing the cognitive deficits as well as the oxidative stress caused by ICV STZ in mice and suggests its potential in age neurodegenerative disorders. In STZ induced memory deficit, there is a decreased activity of glycolytic enzymes resulting in a reduction in acetylcholine level which is intricately associated with cognition. PC showed memory improvement by its potent antioxidant action, is enhancement in CBF and energy metabolism.

Keywords: Soy lecithin, phosphatidylcholine, antioxidant, STZ, CBF

Introduction
The Alzheimer's disease (AD) was first described in 1907 by Alois Alzheimer Neuropath logically it is a progressive neurodegenerative disorder set to become the developed world's largest socioeconomic healthcare burden over the coming decades [1]. AD is thought to affect 4–8% of the population over 65 years of age, with the incidence continuing to increase with increasing age. AD can occur at any age, even as young as 40 years. The rate of occurrence of the disease increases exponentially with age, which means that it occurs very rarely among those 40-50 years old, increases between 60 and 65 years, and is very common over 80 years [2].
Lecithin is mixtures or fractions of phospholipids obtained by physical procedures from animal or vegetable food stuffs; they also include the hydrolysed products obtained through the use of harmless and appropriate enzymes. The final product must not show any signs of residual enzymatic activity. Lecithins may be slightly bleached in aqueous medium by means of hydrogen peroxide. This oxidation must not chemically modify the lecithin phosphatides. Phosphatidylcholine (once given the trivial name 'lecithin') is usually the most abundant phospholipid in animal and plants, often amounting to almost 50% of the total, and as such it is obviously the key building block of membrane bilayers. In particular, it makes up a very high proportion of the outer leaflet of the plasma membrane. Phosphatidylcholine is the major Phospholipid of the brain, liver, plasma and most other tissues [3]. PC is a polar lipid molecule that is a naturally occurring, integral component of the cellular membrane, adding fluidity and strength to cells. PC serves as a source of choline, an important nutrient for liver function and a precursor of the neurotransmitter acetylcholine. Choline is required for the synthesis of Acetylcholine in neurons: treatment that increase brain choline levels also increase the synthesis and release of Acetylcholine [4]. Phosphatidylcholine is important for normal cellular composition and repair. It is the major delivery form of choline, which is a precursor to the synthesis of acetylcholine and other phospholipids.

An increased demand for lecithin as a dietary supplement is also anticipated, as the result of dietary reference intakes being established for choline. PC provides an excellent source of choline, which is essential to every living cell in the body and is one of the main components of cell membranes. Not only is dietary choline important for the synthesis of the phospholipids in cell membranes, it is also necessary for methyl metabolism, cholinergic neurotransmission, transmembrane signaling, and lipid-cholesterol transport and metabolism [5].

Streptozotocin (2-deoxy-2-(3-methy1-3-nitrosoureido) -D-glucopyranose, STZ) is an antibiotic derived from the soil bacteria Streptomycesachromogenes [6] and had been developed as an anticancer agent [7]. STZ is oftenly used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic b cells. Besides its antibiotic and diabetogenic properties, STZ is genotoxic in a variety of assays, including microbial mutagenesis, unscheduled DNA synthesis, micronucleus, chromosomal abberations and sister chromatid exchanges. While parenteral injection of STZ induces diabetes, by damaging pancreatic beta cells possibly through the generation of ROS [8] intracerebroventricular (ICV) injection of streptozotocin (STZ) in rats leads to long-term and progressive deficits in learning, memory, and cognitive performance that is similar to Alzheimer's disease [9].

Materials and methods

Lecithin [2]

Lecithin is the popular and commercial name for a naturally occurring mixture of phosphatides (also called phospholipids or, more recently by biochemists, phosphoglycerides), which varies in color from light tan to dark reddish brown and in consistency from a fluid to a plastic solid. Phospholipids first observed and isolated from egg yolk in 1846 by Maurice Gobley. The term of lecithin was then first used to describe a sticky, orange coloured material, and is derived from Greek *lekithos,* meaning egg yolk.

The term "lecithin" is commonly used as a synonym for phosphatidylcholine (PC), which is the major component of the phosphatide fraction isolated from either egg yolk or soybean and is commercially available in high purity. In commercial practice, however, the term "lecithin" refers to a mixture of different phospholipids (PLs), including "true lecithin" together with other substances such as triglycerides and minor constituents of the lipid fraction that are co-extracted with phosphatides. Lecithin is a complex lipid molecule containing phospholipids – phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and phosphatidylserin (PS), phosphatidic acid (PA). Phosphatidylcholine or 1,2-diacyl-*sn*-glycerol-3-phosphorylcholine is usually the most abundant lipid in the membranes of animal tissues, and it is often a major lipid component of plant membranes, but only rarely of bacteria. With the other choline-containing phospholipid, sphingomyelin, it is a key structural component and constitutes much of the lipid in the external monolayer of the plasma membrane of animal cells especially.

Soy Lecithin [3, 4, 5]

Commercial lecithin is obtained from Soybean (*Glycine max*). Lecithin is a mixture of phospholipids (PL) containing PC, PE, and PI, as well as sphingolipids, FFA, and glyco-lipids. Soybeans are the predominant plant source of lecithin because of their abundant availability and because their lecithin has outstanding functionalities. Soybean lecithin is used as an emulsifier in the food, cosmetics, and pharmaceuticals industries. It typically contains about 18% PC, 14% PE, 9% PI, 5% phosphatidic acid (PA), 2% minor PL, 11% glycolipids, 5% complex sugars, and 37% neutral oil [3].

Lecithin is obtained in the process of degumming of crude soy oil. Crude soy oil contains an average of 1.2-3.2 hydratable compounds, primarily lecithin phosphotides. About 1% live steam or water is added to crude soy oil at about 70°C, in a batch or continuous press. This step hydrates the phospholipid rendering the oil-insoluble and removes triglycerides and other substances. The emulsion then agitated or stirred for 10-60 minutes as the phosphatides hydrates and agglomerate, forming a heavy oil insoluble sludge, which is separated from oil by use of a centrifuge. The sludge coming from degumming centrifuge, a lecithin and water emulsion containing 25-50%water, may then be bleached once or twice to reduce its color from brown to light yellow [3].

Enzymatic hydrolysis of liquid soy lecithin [11-14]

Lecithin and modified lecithin are widely used in the food and pharmaceutical industries as digestible solubilizers and emulsifiers. These emulsifiers are used in a variety of products, e.g. foods, cosmetics, drugs, nutritional supplements and chemicals. The lipase cleaves ester bonds of lipids and produces glycerol and mono- and diglycerides. The phospholipases A_2 cleaves the ester bond of phospholipids at the C_2 position of the glycerol backbone. The resultant lysophospholipids are surface active amphiphilies that contain only one long chain fatty acyl group and a large polar head group consisting of a free hydroxyl on the glycerol backbone and anionic or zwitterionic moiety bonded to the same backbone [13, 14].

lycerol backbone and anionic or zwitterionic moiety bonded to the same backbone [13, 14].

De-oiling of bleached lecithin [15, 16]

De-oiling is required to isolate the phospholipids from the oil. In de-oiled lecithin, the components of soybean oil that are composed only of lipid molecules are removed, but the phospholipids remain. De-oiled lecithin is an emulsifier that exists in powder form. Bleached, de-oiled lecithin is widely used in food industry. The acetone insoluble (A. I.) is the active portion of de-oiled lecithin. On the basis of acetone insoluble it is divided into two types of powder i.e. food powder (min 97% of A. I) and feed powder (min 96% of A. I) [11, 12].

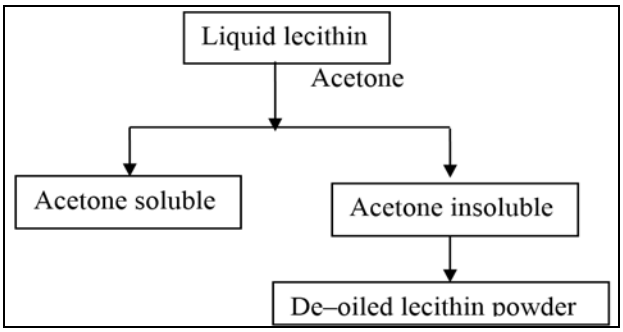


Fig 1

Enrichment of Phosphatidylcholine [17, 18, 19]

Enrichment of Phosphatidylcholine from crude soy lecithin was carried out by– Solvent fractionation and Chromatographic isolation

Enrichment of PC 35

Ethanol can be used for fractionation of PC and PI because of their dissimilar solubility in ethanol. PC is relatively more soluble in ethanol than is PI, ethanol extraction yield a PC enriched fraction. For enrichment of PC 35 bleached liquid lecithin was taken and adds ethyl alcohol in (1: 3.5) ratio the mixture was stirred for 60 min and gets settled for 30 min. After 30 min the mixture was into two phases. The upper ethanol soluble phase, which was PC enriched fraction and lower ethanol insoluble phase PI enriched fraction. The ethanol soluble phase was desolvetized and dried under vacuum to get the desirable viscosity of PC 35. It is further evaluated for the % of PC content by HPTLC and HPLC [17].

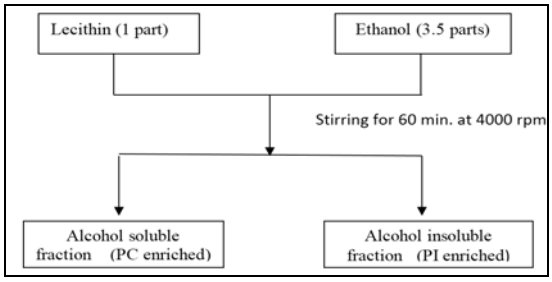


Fig 2

Enrichment of PC 55

For enrichment of PC 55 powder lecithin was treated with 95% ethanol in (1:5) ratio at room temperature. Stirred the mixture for 60 min settled for 15 min. Decant the solvent again add ethanol in (1:3) ratio in remaining powder lecithin again stirred for 60 min again decant the alcohol soluble fraction. The alcohol soluble fraction was pooled together desolvetized and dried under vacuum to get the desirable viscosity of PC 55. It is further evaluated for the % of PC content by HPTLC and HPLC [18].

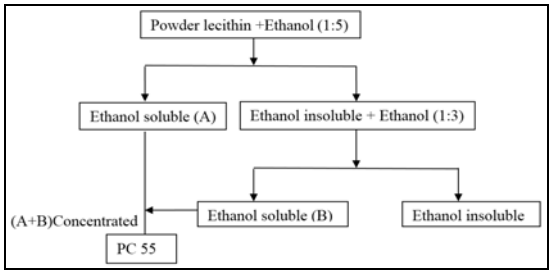


Fig 3

Enrichment of PC 95 by column chromatography

Alkali free alumina oxide (AL_2O_3) gamma grade (γ) is generally employed as chromatographic media. The solvent system absolute ethanol was used for elution of Phosphatidylcholine. Colum having Diameter 1cm to height 3cm ratio was used for the chromatography. The slurry of Alumina (Al_2O_3) was prepared by mixing the adsorbent with sufficient amount of ethanol, poured into the column and washed extensively by the same solvent. A cotton wool was placed at the base of the column and an air bubbles inside the column were removed. Care should be taken that there is no channel created in the bed. Slurry of adsorbent poured gradually and allowed to settle. The solution of PC -55% was poured directly to the column directly to the column. Elute was collected and concentrated to dryness to obtain PC – 95%. It is further evaluated for the % of PC content by HPTLC and HPLC.

Animals: Adult male Swiss albino mice weighing 25–30 g were used. The animals were kept in polyacrylic cage (22.5cm×37.5 cm) and maintained under standard housing conditions (room temperature 24–27°C and humidity 60–65%) with a 12 h light and dark cycle. Food and water were available *ad libitum* but food was not allowed from 1 h prior to and till completion of behavioral study. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), Ministry of Environment And Forests, Government of India, New Delhi.

Materials: Lecithin (liquid), Lecithin (powder), Piracetam (UCB pharma, Mumbai), Streptozotocin (Sigma Aldrich, USA.).

Drug administration

Intracerebral (i.c.) administration of STZ: The ICV cannulationmice were anaesthetized with Ketamine (100 mg/kg) and Xylazine (5mg/ kg) combination. STZ was dissolved in freshly prepared artificial CSF (aCSF) [10] and administered in a volume of 10µl. STZ was injected (0.5 mg/kg) intracerebrally (i.c.) according to the method of Haley and McCormick [11] with a repeat dose 48 h later.

Experimental protocol and administration of PC: For oral administration, PC was suspended in 1.0% (w/v) Tween 80 immediately before administration. To study preventive effect of PC in Morris water maze and EPM, it was administered at doses of 100, 200 and 300 mg/kg for 21 days starting from first dose of STZ.

For experimental protocol dose of PCs was standardized. Various dosed of PCs were given and effective and sub-effective dose of PCs was selected. Animals were randomly divided into 11 groups of six animals each.

Group 1: Sham Operated (SH) (Sham-operated mice wherein the surgery was performed minus drilling of holes and placement of the cannula).

Group2: (SH+ aCSF) artificial cerebrospinal fluid (aCSF) was infused i.c.v. in a volume of 10 µl in ventricle on day 1 and 3.

Group 3: (SH+ Tween 80) Tween 80 was infused orally throughout the study period.

Group 4: (STZ+ aCSF) mice were infused with i.c.v. streptozotocin (0.5 mg/kg) dissolved in aCSF in a volume of 10 µl in ventricle on day 1 and 3.

Group 5: Piracetam (STZ+Piracetam, 100mg/kg, oral) mice were infused with i.c.v. streptozotocin were treated with 100 mg/kg Piracetam for 18 days following 1st streptozotocin infusion.

Group 6-7: PC 35 (STZ+PC 35, 200 and 300 mg/kg) mice were infused with i.c.v. streptozotocin were treated with PCs at doses of 200 and 300 mg/kg, orally respectively for 18 days following 1st streptozotocin infusion.

Group 8-9: PC 55 (STZ+PC 55, 200 and 300 mg/kg) mice were infused with i.c.v. streptozotocin were treated with PCs at doses of 200 and 300 mg/kg, orally respectively for 18 days following 1st streptozotocin infusion.

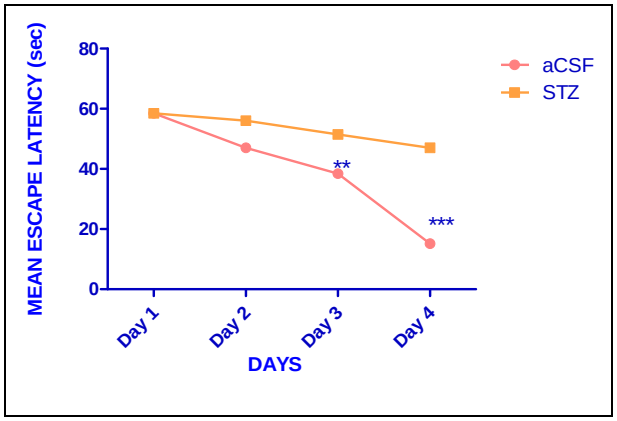
Group 10-11: PC 95 (STZ+PC 95, 200 and 300 mg/kg) mice were infused with i.c.v. streptozotocin were treated with PCs at doses of 200 and 300 mg/kg, orally respectively for 18 days following 1st streptozotocin infusion.
The doses of PCs were selected on the basis of previous studies conducts in laboratory during standardization of doses. The doses of STZ and Piracetam were selected those reported in literature.

Tests employed for learning and memory functions
For the behavioral tests, each group comprised of 6 mice and during behavioral testing only one animal was tested at a given time.
Morris water maze the acquisition and retention of a spatial navigation task was examined using a Morris water maze [12]. Animals were trained to swim to reach a platform in a circular pool (120cm diameter, 50cm height,) located in a darkened test room. The pool was filled with water (26±2 ◦C) to a depth of 30 cm. Four equally spaced points around the edge of the pool were designed as N (North), E (East), S (South) and W (West). A black colored round platform of 8 cm diameter was placed 1 cm below the surface of water in a constant position in the middle of the NE quadrant in the pool; the starting point was in the SW quadrant in all the trials. The water was colored with non-toxic black dye to hide the location of the submerged platform. Trials were given for 5 consecutive days in order to train mice in the Morris water maze. The mice were given a maximum time of 60 s (cut-off time) to find the hidden platform and were allowed to stay on it for 30 s. The experimenter put the mice on platform himself that failed to locate the platform. The animals were given a daily session of 3 trials per day. Latency time to reach the platform was recorded in each trial. Mean latency time of all three trials is shown in the results. A significant decrease in latency time from that of 1st session was considered as a successful learning [13].

Elevated plus Maze
Acquisition and retention of memory processes was assessed using elevated plus maze on day 18, 19 of STZ injection. Elevated plus maze consists of two opposite open arms (50 X 10 cm), crossed with two closed arms of the same dimensions with 40 cm high walls. The arms are connected by a central square (10 X 10) and all of these are elevated 70 cm from the floor. On day 18 after i.c.v. injection, rats were placed individually at one end of the open arm, facing away from the central square. The time taken for the rat to move from the open arm and enter into one of the closed arms was recorded as "initial transfer latency" (ITL). The animal was allowed to explore the maze for 30 s after recording ITL and returns to its home cage. Then, 24 h after ITL, the rat was placed again on the open arm and the retention latency was noted as "retention transfer latency" (RTL) [14, 9].

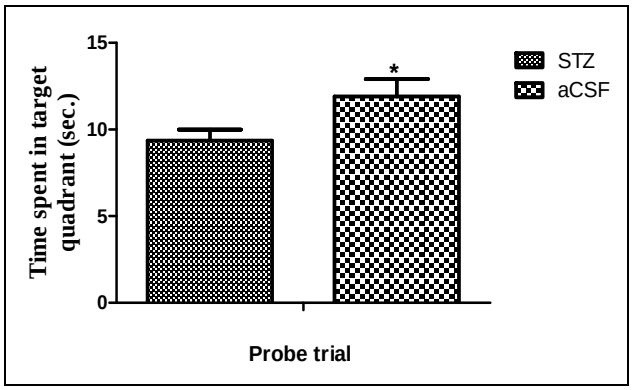
Spontaneous locomotors activity
Each animal was tested for spontaneous locomotors activity on day 14, 15, 16 and 17 following 1st i.c.v. streptozotocin infusion. Each animal was observed over a period of 5 min in a square closed arena equipped with infrared light sensitive photocells using a digital photoactometer (INCO, India) [15].

Results
Effect of ICV STZ on acquisition in MWM
STZ at 0.5mg/kg, I.C.V significantly increased escape latency in acquisition training as compared to aCSF group on two successive days except first and second day. Application of two-way ANOVA showed interaction between variables viz, STZ treatment and acquisition days [F (3, 40) = 6.63, $P<0.05$]. Application of post hoc bonferroni tests revealed that STZ do not show any significant difference in escape latency as compared to acsf. Two way ANOVA revealed a main effect of treatment [F (1, 40) = 26.80, $P<0.0001$] and acquisition days [F (3, 40) = 20.12, $P<0.0001$].



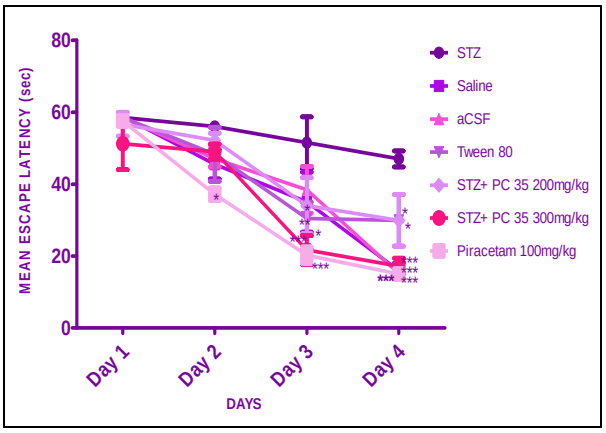
Graph 1: Effect of STZ in MWM

Effect of STZ on retrieval (probe trail) in MWM:-
STZ at 0.5mg/kg, I.C.V significantly decreased time spend in target quadrant in Probe Trial session, i.e on 18th day of activity, as compared to aCSF group. Mean Time spent in target quadrant± S.E.M was 26.5 ± 2.82 sec and 11.833 ± 2.12 sec for aCSF and STZ respectively aCSF groups shows significant difference when compared with STZ treated mice.



Graph 2: Effect of STZ on Probe trial in MWM (n=6)

Effect of PCs, Piracetam and ICV STZ on mice in MWM
1. Effect of PC 35 in MWM:-
Prior administration of PC 35 300mg/kg significantly decreased escape latency as compared to Saline group on 3rd and 4th day but no significant result was obtained for first and second day. Prior administration of Piracetam 100mg/kg significantly decreased escape latency on day second, third and fourth and that of PC 35 200mg/kg on day third and fourth respectively as compared to STZ group. Application of two way ANOVA showed significant interaction between variables viz; STZ treatment and acquisition days [F (18, 140) = 2.02, $P<0.05$]. Post Hoc Bonferroni Multiple Comparison Tests revealed that prior administration of PC 35 significantly decreased escape latency two way ANOVA revealed a main effect of treatment [F (6, 140) = 9.30, $P<0.0001$] and acquisition days [F (3, 140) = 77.51, $P<0.0001$].



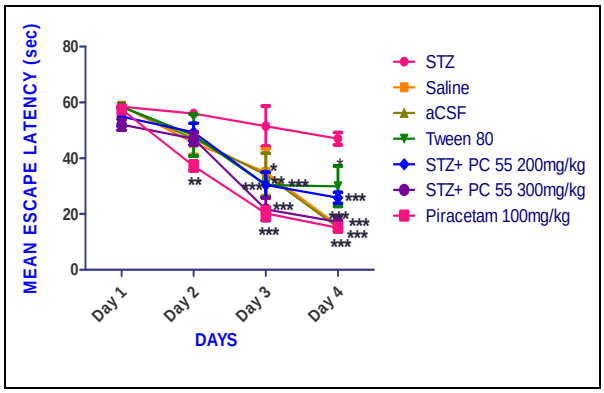
Graph 3: Effect of PC 35 (200 and 300 mg/kg) on memory performance in MWM. Escape latencies were observed on day 14th, 15th, 16th, 17th I.C.V administration of STZ on 1st and 3rd day. Values are represented as mean ± SEM and analyzed by two way ANOVA (n=6), **P*<0.05, ***P*<0.01, ****P*<0.001 considered as statistically significant as compared with STZ group.

2. Effect of PC 35 on retrieval (probe trail) in MWW

Further with PC 35 200 and 300 mg/kg mice there was increase in time spent in target quadrant during probe trail session i.e. on day 18 of activity. Mean Time spent in target quadrant± S.E.M was 11.953± 0.389 sec and 13.20± 0.976 sec for PC 35 200 and 300 mg/kg respectively. PC 35 group's shows significant difference when compared with STZ treated mice (Graph 6).

3. Effect of PC 55 in MWM

Prior administration of PC 55 300mg/kg significantly decreased escape latency as compared to Saline group on 3rd and 4th day but no significant result was obtained for first and second day. Prior administration of Piracetam 100mg/kg significantly decreased escape latency on day second, third and fourth and that of PC 55 200mg/kg on day third and fourth respectively as compared to STZ group. Application of two way ANOVA showed significant interaction between variables viz; STZ treatment and acquisition days [F (18, 140) = 2.26, *P*<0.05]. Post Hoc Bonferroni Multiple Comparison Tests revealed that prior administration of PC 55 significantly decreased escape latency two way ANOVA revealed a main effect of treatment [F (6, 140) = 11.54, *P*<0.0001] and acquisition days [F (3, 140) = 100.52, *P*<0.0001].



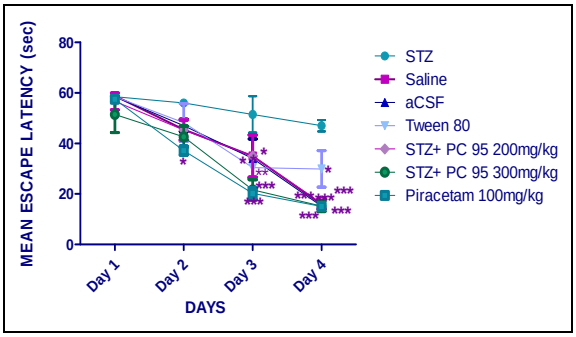
Graph 4: Effect of PC 55 (200 and 300 mg/kg) on memory performance in MWM. Escape latencies were observed on day 14th, 15th, 16th, 17th I.C.V administration of STZ on 1st and 3rd day. Values are represented as mean ± SEM and analyzed by two way ANOVA (n=6), **P*<0.05, ***P*<0.01, ****P*<0.001 considered as statistically significant as compared with STZ group.

4. Effect of PC 55 on retrieval (probe trail) in MWW

Further with PC 55 200 and 300 mg/kg P.O mice there was increase in time spent in target quadrant during probe trail session i.e. on day 18 of activity. Mean Time spent in target quadrant ± S.E.M was 11.98 ± 0.4053 sec and 13.26± 0.531 sec for PC 35 200 and 300 mg/kg respectively. PC 35 group's shows significant difference when compared with STZ treated mice (Graph 6).

5. Effect of PC 95 in MWM

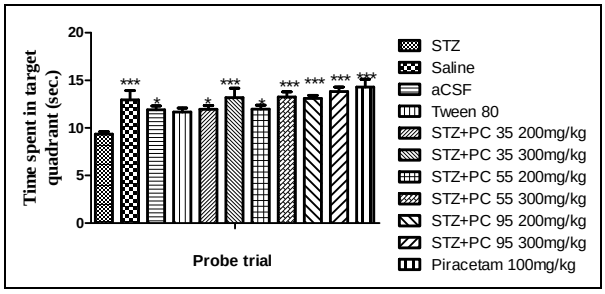
Prior administration of PC 95 300mg/kg significantly decreased escape latency as compared to Saline group on 3rd and 4th day but no significant result was obtained for first and second day. Prior administration of Piracetam 100mg/kg significantly decreased escape latency on day second, third and fourth and that of PC 95 200mg/kg on day third and fourth respectively as compared to STZ group. Application of two way ANOVA showed significant interaction between variables viz; STZ treatment and acquisition days [F (18, 140) = 1.75, *P*<0.05]. Post Hoc Bonferroni Multiple Comparison Tests revealed that prior administration of PC 95 significantly decreased escape latency two way ANOVA revealed a main effect of treatment [F (6, 140) = 9.91, *P*<0.0001] and acquisition days [F (3, 140) = 83.90, *P*<0.0001].



Graph 5: Effect of PC 55 (200 and 300 mg/kg) on memory performance in MWM. Escape latencies were observed on day 14th, 15th, 16th, 17th I.C.V administration of STZ on 1st and 3rd day. Values are represented as mean ± SEM and analyzed by two way ANOVA (n=6), **P*<0.05, ***P*<0.01, ****P*<0.001 considered as statistically significant as compared with STZ group.

6. Effect of PC 95 on retrieval (probe trail) in MWW

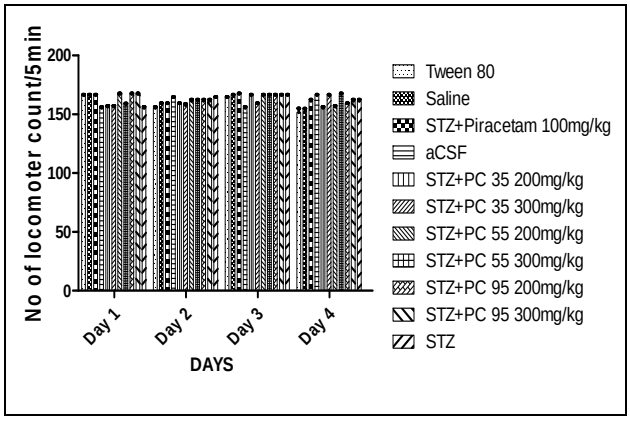
Further with PC 95 200 and 300 mg/kg P.O mice there was increase in time spent in target quadrant during probe trail session i.e. on day 18 of activity. Mean Time spent in target quadrant ± S.E.M was 13.11± 0.319 sec and 13.83± 0.463 sec for PC 95 200 and 300 mg/kg respectively. PC 35 group's shows significant difference when compared with STZ treated mice.



Graph 6: For probe trial data was analyzed by One Way ANOVA followed by Dunnet test, Values are represented as mean ± S.E.M, ***P*<0.01, **P*<0.05 considered as statistically significant as compared to STZ group.

7. Effect of PCs, Piracetam and STZ on locomotors activity

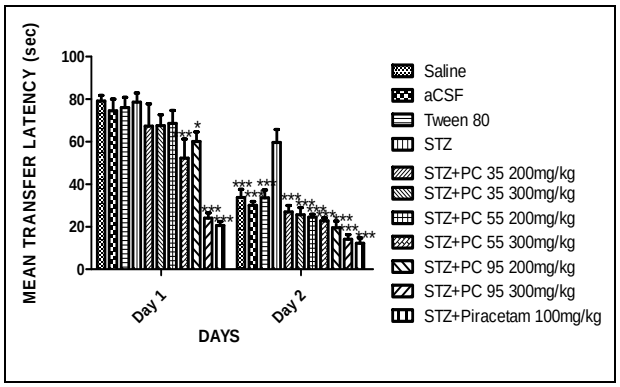
In present series of experiment mean scores of locomotors activity for each mice was relatively stable and showed no significant difference, STZ injected mice did not cause any alteration in locomotors activity compared with aCSF injected mice on days 14, 15, 16and 17 of activity. Two ANOVA revealed that there was no significant difference in drug treatment.



Graph 7: For locomotors activity, no statistically significant data obtained when data was analyzed by Two ANOVA.

Effect of PCs, Piracetam and ICV STZ on mice in EPM

Prior administration of Piracetam 100mg/kg significantly increase initial transfer latency and that of PC 95 200,300mg/kg and PC 55 300mg/kg significantly increase initial transfer latency respectively as compared to STZ group. On day second all group showed significantly increase retention transfer latency respectively as compared to STZ group. Application of two way ANOVA showed significant interaction between variables viz; STZ treatment in two days [F (10, 110) = 4.76, P<0.001]. Post Hoc Bonferroni Multiple Comparison Tests revealed that prior administration of PCs significantly increase transfer latency two way ANOVA revealed a main effect of treatment [F (10, 110) = 22.79, P<0.0001] and acquisition days [F (1, 110) = 284.49, P<0.0001].



Graph 8: Performance of PCs treated and non-treated mice on Elevated plus maze learning and memory task. Mean scores of first and second day transfer latency (TL) of each group of mice are expressed as ±SEM (n=6) *P<0.05, ***P<0.001 was statistically significant analyzed by Two way ANOVA.

Discussions

It is a well-established fact that ICV injection of STZ is characterized by a progressive deterioration of learning, memory and cerebral glucose and energy metabolism and this may provide an appropriate and relevant experimental model of SAD [16, 17]. In the present study, STZ at a dose of 0.5 mg/kg was used. This dose has been shown not to cause any change in the peripheral blood glucose level, although this dose induces a significant cognitive impairment in animals [17]. There is strong evidence for the fact that inflammatory processes are associated with the pathophysiology of Alzheimer's disease and that treatment with NSAIDS reduces the risk for Alzheimer's disease [18]. Since free radical generation is also associated with cognitive impairment in ICV STZ model of SAD in rats [17], therefore, PCs in this way could attenuate the observed behavioral deficits in this study. Furthermore, it has recently been demonstrated that ICV STZ in rats could also lead to increased expression of beta amyloid in the rat brain which itself may enhance inflammatory processes within the central nervous system [19]. ICV injection of STZ, which inhibits insulin receptor function, develops progressive behavioral deficits as well as biochemical changes and neuronal degeneration in rats that is very similar to SAD [20]. It has been suggested that ICV STZ may cause neuronal damage independent of its action on glucose metabolism [21]. In this respect, STZ induces specific damage to axons and myelin in some brain regions including the fornix, anterior hippocampus and periventricular area that are essential for learning and spatial memory. Therefore, it seems likely that STZ does not induce learning deficits only by impairing glucose utilization [22] through an action on brain insulin receptor function [23]. Although the mechanism of action of STZ on myelin is not yet known, it probably involves the induction of oxidative stress, to which myelin is particularly vulnerable [21]. Evidence of lipid peroxidation in whole brain homogenates was provided by the finding of an increase in malondialdehyde and a decrease in glutathione 3 weeks after ICV injection of STZ [17]. It is possible that the protective effect of PCs in this study has been through its effects on these processes [24].

Apart from insulin receptor signalling dysfunction ICV STZ also leads to direct damage to the septohippocampal system. This was supported by reduced choline acetyl transferase (ChAT) activity in hippocampus [25], reduction in the weight of septum by more than 40% [26], decrease in the transport of nerve growth factor (NGF) from hippocampus to septum [27], microglial activation and specific damage to myelinated tracts in the fornix through generation of oxidative stress, thereby disrupting connections between the septum and hippocampus [21]. Neuroinflammation plays an important part in the pathogenesis of Alzheimer's disease and cognitive deficits in AD are attributed to recruitment and activation of microglia and astrocytes [28] which release cytokines, reactive oxygen species (ROS) and nitric oxide (NO) [29]. Oxidative damage to the rat synapse in these regions of brain has been previously reported to contribute to cognitive deficits [30]. Oxidative and nutritive stresses are the crucial factors deciding behavioral changes and finally cell death in AD. Some study shows that in chronically hyperglycemic rats with type 1 diabetes mellitus, the expression patterns of PKC isoforms in the rat brain are affected in a complex manner [31]. In STZ induced memory deficit, there is a decreased activity of glycolytic enzymes resulting in a reduction in acetylcholine level [22, 32] which is intricately associated with cognition.

Several studies have been done with phosphatidylcholine to investigate its effects on memory. The results of the studies have not been consistent. Some have shown positive responses [33, 34], while others showed no difference in memory

or learning after lecithin administration [35]. Phosphatidylcholine may help some with tardive dyskinesia, a neurological disorder characterized by defective cholinergic nerve activity.

Effect on MWM, EPM

In present study we evaluated drugs for their antioxidant properties in the streptozotocin (i.c.)-induced model of AD, because free radical generation is a major component of neurodegeneration along with memory impairment in this model [16, 36]. Streptozotocin (i.c.) injected twice 48 h apart in mice by i.c. route resulted in a persistent significant deficit in performance in Morris water maze and Elevated plus maze tests 14 days after the first dose. Administration of aCSF by i.c. route in a similar manner to streptozotocin (i.c.) did not hinder learning in mice subjected to these tests. The Morris water maze test is used to test spatial memory by observing the latency to reach a hidden platform. A decrease in latency time in repeated trials demonstrates intact learning and memory function [37]. Streptozotocin (i.c.)-treated mice did not show this decline in the latency time, whereas PCs treatment of streptozotocin (i.c.)-treated mice decreased the time to reach the hidden platform. Therefore, PCs showed their efficacy in this streptozotocin (i.c.) model of dementia in Morris water maze, Elevated plus maze and Novel object recognition task test. The effectiveness of PCs in this streptozotocin (i.c.) model of dementia also indicates the possibility that these anticholineserase drugs have antioxidant activity because the streptozotocin model is based predominantly on free radical generation.

Results show that PC contains the phenolic, flavonoids, carbohydrates and steroids contents. The results suggest that PC 95 is a more potent scavenger of NO than other samples. PCs possess antioxidant activity evidenced by an *in-vitro* reductive ability. Phenolic antioxidants such as flavonoids, tannins, scavenge radicals and thus they are viewed as promising therapeutic drugs for free radical mediated pathogenesis [38]. The salient findings of this study indicated that chronic treatment with PCs, in mice with ICV administered STZ, caused a significant improvement in the memory performance tasks as assessed in Morris water maze. In Standardisation for the dose of PCs at different doses (100, 200 and 300mg/kg), orally, the mean escape latency was decreased while retention time was increased dose dependently. In conclusion 200 and 300mg/kg per oral dose of PCs effectively enhance memory. On this basis we selected 200mg/kg as sub effective dose and 300mg/kg as an effective dose further activity. The study demonstrates the effectiveness of PC in preventing the cognitive deficits as well as the oxidative stress caused by ICV STZ in mice and suggests its potential in age and age-related neurodegenerative disorders. In summary the result of the present study suggest that PCs produces Nootropic effect in STZ induced memory impairment in MWM. It showed this effect in dose dependent manner. The salient findings of this study indicated that chronic treatment with PCs, in mice with ICV administered STZ, caused a significant improvement in the memory performance tasks as assessed in EPM and Actophotometer when compared with standard drug.

Conclusion

Recently, there has been an exponential growth in interest in natural antioxidants, in particular plant-derived antioxidants from aromatic, medicinal and edible plants. Therefore, samples were screened for antioxidant properties in a battery of *in-vitro* assays. The sample demonstrated a wide range of antioxidant properties, some of which were relevant to AD therapy. These components are proven antioxidants; therefore, it is possible they are contributing to the activity of the samples. The PCs contains good amount of antioxidants, which can be used for treatment of oxidative stress and other diseases. PC contains the phenolic, Flavonoids, carbohydrates and steroids contents. Phenolic antioxidants such as flavonoids, tannins, scavenge radicals and thus they are viewed as promising therapeutic drugs for free radical mediated pathogenesis.

The study demonstrates the effectiveness of PC in preventing the cognitive deficits as well as the oxidative stress caused by ICV STZ in mice and suggests its potential in age and age-related neurodegenerative disorders. In summary the result of the present study suggest that PCs produces Nootropic effect in STZ induced memory impairment in MWM.

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