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THE STUDY ON NEUROPROTECTIVE AGAINST ALCL3 INDUCED TOXICITY AND ENHANCED LEARNING AND ITS MORRIS WATER MAZE TEST IN RATS.

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Abstract:

Objective: The current study aimed at the investigation of the effectiveness of ethanolic and methanolic extract of *Polygonum glabrum* in aluminum chloride-induced Alzheimer's disease in experimental rats.

Methods: The behavioral parameters evaluated by following methods such as Morris water maze test, radial arm maze test, and active avoidance test. Biochemical parameters were also estimated such as acetylcholine and acetylcholine esterase.

Results: *Polygonum glabrum* extract was instituted to be neuroprotective against AlCl3-induced toxicity. Enhanced learning and memory were allied to the ingestion of extract in rats.

Conclusion: Based on these current findings, it is suggested that lowering Aβ is an unproven strategy, and it may be time to refocus on other targets for the treatment of this disease, includingpathological forms of tau.

Introduction:

Neurogenerative Disorders:

Neurodegenerative diseases are heterogeneous grouping of genetic disorders that are designated by means of loss of neuronal function, structure and in general direct to neuronal loss. These diseases may perhaps consequence directly from specific degeneration of neuronal population or not directly from changes in support iveglial cells. These types of diseases are characterized by means of an abnormal proteins buildup or other biological materials build up outside or within the neurons. These aggregations take diverse structures and consequence in neurofabrillary tangles in Alzheimer's disease, glycogen and polyglucosan bodies in Lafora disease, Lewy bodies in Parkinson's disease.

Alzheimer's Disease:

Alzheimer's disease is consideration to be in responsible of around 60% of all mental disorder in older adults or moderately aged [1] and influence in more than of 5 million Americans, a number assessed to augmentation to 7.7million by means of 2030.Alzheimer's disease is caused because of aggregation of misfolded proteins build up of deposits of fibrillary amyloid in selective areas of central nervous system. Alzheimer's disease leads in memory loss,unusual behavior,personality changes, and loss of the capability to thinking. Early disease exhibits short term memory loss, fail to retain information names and addresses, not able to learn novel information, mood swings, as the circumstance develops, transform become more prominent and persons even forgets way to home.

Methodology:

The aerial parts of fresh and new medicinal plants collected. To get relieve of adhered dirt; aerial parts were rinsed and cleaned by means of distilled water subsequently blotted
thoroughly and dehydrated by means of shade in its consign of sun light. The shadily dried aerial parts were crushed by means of a commercially available mixer. The obtained medicinal plant powder was auxiliary filter to get fine powder and engaged for extraction by means of solvents. Almost 100 g of the crushed plant medicinal aerial parts was kept for Soxhlet extraction by means of 1000 ml solvents, Ethanol and Methanol. This accurate cycle was repetitive again and again, for hours to a hardly many a days, till the color of the solvent washing out away in the siphon of the soxhlet. The extract was resolute under condensed pressure (P) and stock up in refrigerator proceeding to commencing for advanced utilizations.

EXPERIMENTAL ANIMALS:

Each rat that weighed in in the midst of 180-200 gm was kept back disconnectedly. The animals were just kept back for 2 days to 7 days to get make use of to the conditions of animal area. They were handling in prescribed laboratory setting of temperature 22±2^{o}c, 70% humidity and cycles of 12 hours light and dark. And animals fed with standard diet of pellet and adequate valve water.

Acute Toxicity

Prior to commencing animal study, the acute toxicity study investigations were done to establish effective dose of test compounds. Based on acute toxicity results, the lead extracts were tested in appropriate animal models.[130]

In-Vivo Study

The behavioral screening was done by following methods[131-138]

Morris water maze test

Morris water maze test was performed for assessment of the retention of working (as a reference) and spatial memory in wistar rats. The water maze contains of a circular tank (150 cm in diameter and 40 cm in height). Water pool was alienated into 4 equally spaced quadrants all along the circumference of the pool. A flee platform (10 cm in diameter) submerged 2 cm beneath the water surface was positioned in NW quadrant. Rats were trained to situate the hidden platform at a fixed spot in NW quadrant. All rats were kept to 1 session of 4 trials per day. During each trial, the animal was positioned in each quadrant to remove quadrant effects. All rats were left in the platform for 30 seconds and then removed and dried by using towel[139]. Rats failing to locate the platform within 60 s were directed to the platform. 24 h after previous training, escape platform was taken away and probe trial was conducted. The cutoff time for animal to swim was set to at 60 s prior to the finish of

session. Time elapsed in escaping to the NW quadrant, i.e. escape latency time (ELT) was measured all through the trials of retention.

Fig 1: **Morris water maze test**

Statistical analysis of data:

Results were characterized as mean ± S.E.M. The statistical variation amid the groups was computed in characteristic of ANOVA with mean ± S.E.M. The discrepancy was considered significant if P< 0.05.

Results

1) **Percentage yield of ethanolic and methanolic extract of *Polygonum glabrum***

Table 7: Percentage yields of extracts

S no	Solvent	Percentage Yield
1.	Ethanol	12.4%
2.	Methanol	10.5%

2) Phytochemical component observed in ethanolic and methanolic extract of *Polygonum glabrum*

Table 8: Particulars of qualitative phytochemical assessment

S. No.	Test	Methanolic Extract	Ethanolic Extract
1	**Alkaloids**		
	Mayer's test	+	+
	Dragondraffs Test	+	+
	Hager's Test	+	+

	Wagner's test	+	+
2	**Carbohydrates**		
	Mohlish's test	+	+
3	**Reducing Sugars**		
	Fehling's test	+	+
	Benedicts Test	+	+
4	**Saponins**		
	Foam test	-	-
	Forth Test	-	-
5	**Phytosterols**		
	Salkowski Test	+	+
	Liberman Burchard's Test	+	+
6	**Phenolics**		
	Ferric chloride test	-	-
	Lead acetate test	-	-
7	**Tannins**		
	Ferric chloride test	+	+
8	Flavones and Flavonoids		
	Lead Acetate Test	+	+
	Alkaline Reagent Test	-	+
9	Glycosides		
	Keller killliani test	+	+
10	Proteins and amino Acids		
	Ninhydrintest	+	+
	Biuret test	+	+
11	Terpenoids		
	Salkowskis Test	-	-
12	Fixed oils and fats		
	Spot test	-	-
	Saponification Test	-	-
13	Gum and Mucilages		
	Ruthenium Red Solution	+	+

(+)POSITIVE (-) NEGATIVE

3) ACUTE TOXICITY TESTING

The Acute toxicity valuation performed on animals exposed that the *Polygonum glabrum* Methanolic and ethanolic extract was in safe and sound even at amount of 2 gram/kgb.w. The reasonably accurate and precise LD_{50} is may possibly is>2 gram/kg b.w. And hitherto supplementary not any lethality or any class of toxic retort and or moribund class of state was observed till the finish of the extent of investigational research.

Table 9: Acute Toxicity Assessment

S.no.	Code	Toxicity		Time of Death	Observation										
		Onset	Stop		Skin Color	Eyes	Resp	CNS	Tre	Con	Sali	Diah	Sleep	Leth	
1.	MCG	x	X	x	X	x	x	X	x	x	x	X	X	X	
2.	MCG	x	X	x	X	x	x	X	x	x	x	X	X	X	

(TRE-Tremor, CON-Convulsions, SALI- Salivation, Diah - Diarrhea, LET-Lethargy)

× = Negetive Ø = Positive

4) Effect of ethanolic and methanolic Extract of *Polygonum glabrum* On Behavioral activity by morris water maze test.

In relevant case of the of ethanolic and methanolic extract of *Polygonum glabrum* effectiveness on escape latency time made by means of morris water maze test, the ethanolic and methanolic extract exposed considerably decline in escape latency time. Ethanolic extract is more effective as comparison with methanolic extract.

Table 10: Effect of ethanolic extract of Polygonum glabrum effectiveness on escape latency time made by means of morris water maze test

GROUPS	Escape Latency Time (Sec)		
	7th day	**14th day**	**21st day**
Normal	4.85 ± 0.37	5.23 ± 0.29	5.34 ± 0.68
Disease Control ($AlCl_3$ 300mg/kg)	5.14 ± 0.19[a]	15.86 ± 0.76[a]	22.18 ± 0.72[a]
Standard (Rivastigmine 0.3 mg/kg I.P.)	4.87 ± 0.39	11.72 ± 0.48	7.39 ± 0.64***
EPG (100mg/kg)	4.97 ± 0.60	13.54 ± 0.32	10.22 ± 0.89***
EPG (200 mg/kg)	4.91 ± 0.34	12.98± 0.38	9.43 ± 0.63***
EPG (400 mg/kg)	4.89± 0.29	12.12± 0.27	7.84 ± 0.57***

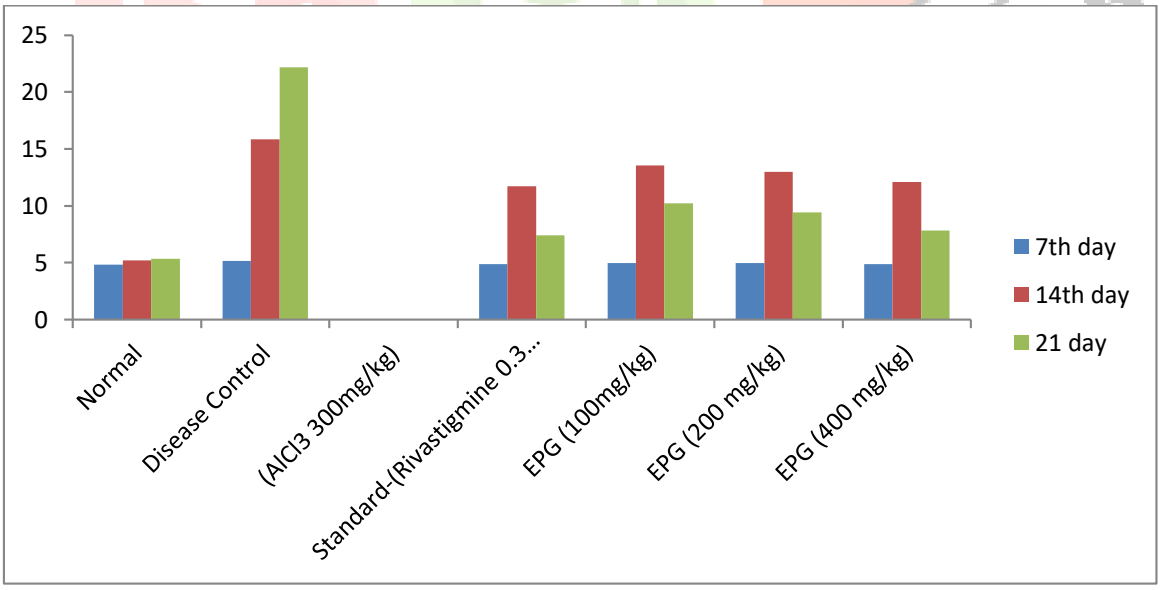


Fig 1: **Effect of ethanolic extract of Polygonum glabrum effectiveness on escape latencytime made by means of morris water maze test**

Data represented as mean ± S.E.M values of 6 animals each. *p<0.05, **p<0.01 (Dunnett t-test);control was compared with extract and standard treated groups were compared

Table 11: Effect of methanolic extract of Polygonum glabrum effectiveness on escapelatency time made by means of morris water maze test

GROUPS	**Escape Latency Time (Sec)**		
	7th day	**14th day**	**21st day**
Normal	4.87 ± 0.34	5.12 ± 0.27	5.47 ± 0.63
Disease Control ($AlCl_3$ 300mg/kg)	5.32 ± 0.21[a]	16.01 ± 0.79[a]	22.18 ± 0.66[a]
Standard(Rivastigmine 0.3 mg/kg I.P.)	4.89 ± 0.43	12.67 ± 0.48	7.43 ± 0.61***
MPG (100mg/kg)	5.12 ± 0.65	14.72 ± 0.39	11.37 ± 0.77***
MPG (200 mg/kg)	4.93 ± 0.39	13.59± 0.37	10.12 ± 0.54***
MPG (400 mg/kg)	4.91± 0.34	12.82± 0.52	8.45 ± 0.68***

Data represented as mean ± S.E.M values of 6 animals each. *p<0.05, **p<0.01 (Dunnett t-test); control was compared with extract and standard treated groups were compared.

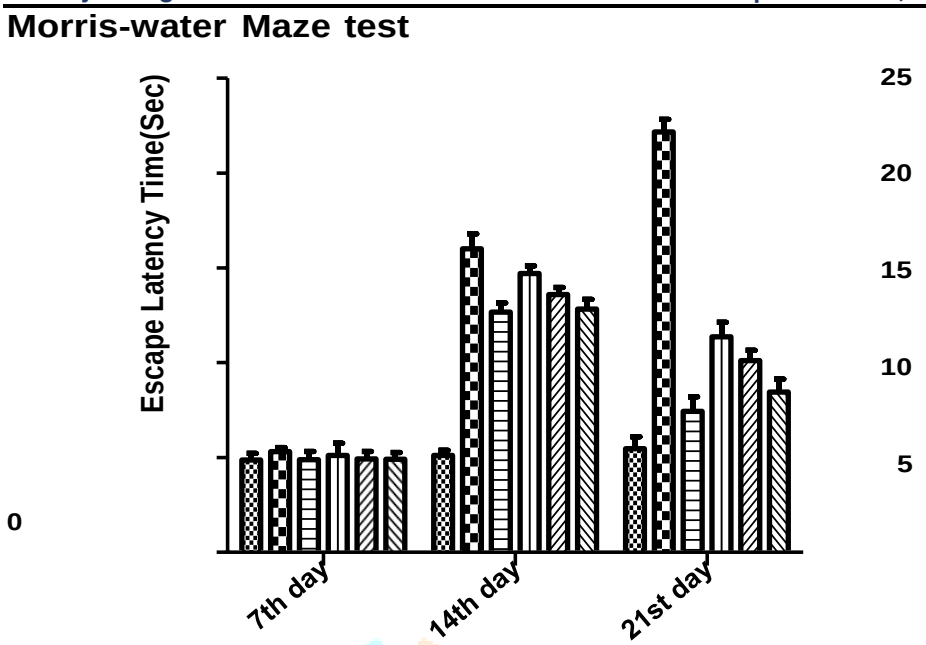
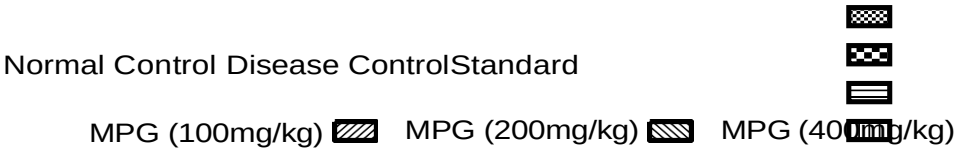


Fig 43: **Effect of methanolic extract of Polygonum glabrum effectiveness on escape latency time made by means of morris water maze test**



Data represented as mean ± S.E.M values of 6 animals each. *p<0.05, **p<0.01 (Dunnett t-test); control was compared with extract and standard treated groups were compared.

In relevant case of the of ethanolic and methanolic extract of Polygonum glabrum effectiveness on time lapse by means of radial arm maze test, the ethanolic and methanolic extract exposed considerably decline in time lapse. Ethanolic extract is more effective as comparison with methanolic extract.

Table 16: Effect of ethanolic extract of Polygonum glabrum effectiveness acetylcholine and acetylcholineestersase levels

GROUPS	**Ach (µmol/mg protein)**	**AchE(unit/mg protein)**
Normal	5.65 ± 0.15	0.54 ± 0.04
Disease Control ($AlCl_3$ 300mg/kg)	0.87± 0.05	0.82 ± 0.02
Standard (Rivastigmine 0.3 mg/kg I.P.)	5.4± 0.17	0.56 ± 0.03
EPG (100mg/kg)	2.7 ± 0.23	0.75 ± 0.05
EPG (200 mg/kg)	3.4 ± 0.21	0.63± 0.04
EPG (400 mg/kg)	4.9± 0.29	0.59± 0.06

Data represented as mean ± S.E.M values of 6 animals each. *p<0.05, **p<0.01 (Dunnett t-test);control was compared with extract and standard treated groups were compared

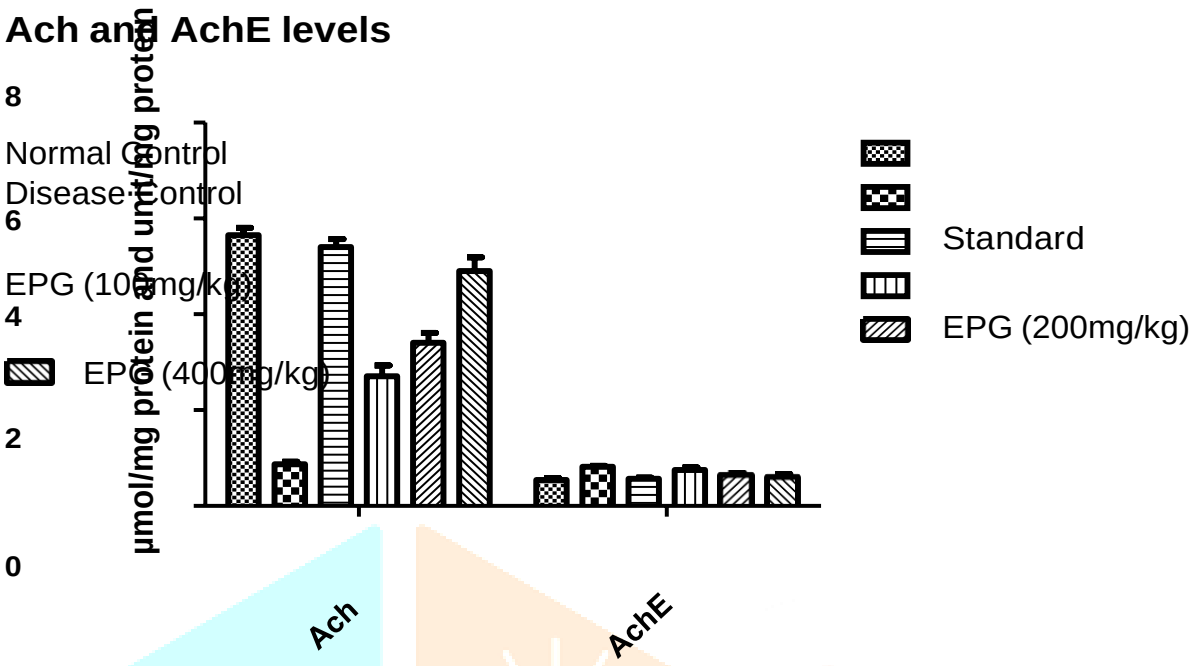


Fig 4 : **Effect of ethanolic extract of Polygonum glabrum effectiveness acetyl choline andacetyl choline estersase levels**

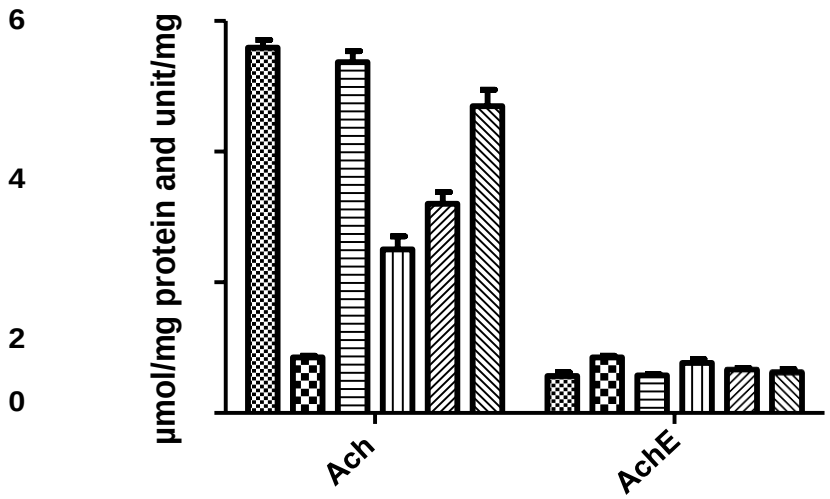
Data represented as mean ± S.E.M values of 6 animals each. *p<0.05, **p<0.01 (Dunnett t-test);control was compared with extract and standard treated groups were compared

Table 17: Effect of methanolic extract of Polygonum glabrum effectiveness acetylcholineand acetylcholine estersase levels

GROUPS	Ach (µmol/mg protein)	AchE(unit/mg protein)
Normal	5.59 ± 0.12	0.57 ± 0.05
Disease Control ($AlCl_3$ 300mg/kg)	0.85± 0.03	0.85 ± 0.03
Standard (Rivastigmine 0.3 mg/kg I.P.)	5.37± 0.17	0.58± 0.02
MPG (100mg/kg)	2.5 ± 0.2	0.77 ± 0.06
MPG (200 mg/kg)	3.2 ± 0.18	0.66± 0.03
MPG (400 mg/kg)	4.7± 0.25	0.61± 0.05

Data represented as mean ± S.E.M values of 6 animals each. *p<0.05, **p<0.01 (Dunnett t-test);control was compared with extract and standard treated

Ach and AchE levels



Data represented as mean ± S.E.M values of 6 animals each. *p<0.05, **p<0.01 (Dunnett t-test);control was compared with extract and standard treated

Discussions:

Results obtained from water maze test demonstrated dependable lessen in the time required to find the platform. The aluminum treated rats showed high fluctuation in the time required to reach the platform from one day to another. The declining rate of the time needed to reach the platform was relatively steady for the control, standard and group treated with extracts. These out comes guide us to guesstimate that extract may exert its effect by means of managing the lessening rate of time. The fluctuation in the time required to find the platform showed by means of AlCl3 treated rats was lessened by means of the ingestion of PG. Suggesting that, although the effect of PG was not revealed by means of monitoring the time needed to find the platform, yet, it was demonstrated that PG effect was resulted to the waning rate in the time and reverting the fluctuation produced by means of AlCl3. A high correlation was found between the radial maze test and the avoidance response in PG treated groups. The correlations specify that both radial arm maze and active avoidance test possibly will assess memory dispensation in a definite region of rat's brain. As a part of model of active avoidance (AA), demonstrated 2-fold lessening correct reactions from the 7^{th} to the 21^{st} day of assessment in AlCl3-treated animals as compared with the group of control.

Conclusion:

Polygonum glabrum extract was instituted to be neuroprotective against $AlCl_3$ induced toxicity. Enhanced learning and memory was allied to ingestion of extract in rats. Al overload, AChE hyperactivity are responsible for alzheimers disease which are neutralized or reduced with treatment of extract, which might be due to the synergistic action of its active constituents. Ethanolic extract was shown slightly higher efficacy as compared to methanolic extract. However extensive research is needed to validate the anti-alzheimeric effect of extract active components against a variety of models of AD, prior to entering into the clinical trials.

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