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Pharmacological Evaluation of Transcranially Applied Lignocaine Gel as a Novel Amnesic Agents on Rodents

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ABSTRACT : Anterograde amnesia means a loss of the ability to create new memories after the event that caused the amnesia, leading to a partial or complete inability to recall the recent past, while long term memories from before the event remain intact. Lignocaine belongs to a group of medicines known as local anaesthetics. It works by temporarily blocking the pathway of pain signals along nerves. Apart from the established routes of drug administration practiced in modern medicine, *Ayurvedic* system of medicine uses a special route which involves oil therapies to the head. These therapies are used for centuries to treat diseases of the central nervous system. In present study Lignocaine gel 2% is used to evaluate, it causes anterograde amnesia after transcranial application; a brain targeted drug delivery. Amnesic effect was evaluated using rod walking test, locomotor activity test, elevated plus maze test, water maze test, pole climbing test and pole climbing test on trained animal. In conclusion, based on the findings of the present study Lignocain TC application is effective in producing amnesia when it evaluated on different learning and memory evaluation model. This can be used as amnesic inducing agent upon transcranial application to the rodent

KEYWORDS **:** Transcranial route (TCR), Amnesia, Pole climbing test, learning and memory, water maze test,

I. INTRODUCTION:

Anterograde amnesia is a loss of the ability to create new memories after the event that caused the amnesia, leading to a partial or complete inability to recall the recent past, while long-term memories from before the event remain intact.One cause is benzodiazepine drugs, such as midazolam, flunitrazepam, lorazepam etc. which are known to have powerful amnesic effects. This has also been recorded in non-benzodiazapine sedatives which act on the same set of receptors, such as Zopiclone . Another cause is a traumatic brain injury in which damage is usually done to the hippocampus or surrounding cortices. It can also be caused by shock or an emotional disorder. The researcher scopolamine is use as amnesic inducing agent.However, Lignocaine belongs to a group of medicines known as local anaesthetics. It works by temporarily blocking the pathway of pain signals along nerves. Pain is caused by the stimulation of pain receptors at the ends of nerves. The stimulation causes sodium to enter the nerve ending, which causes an electrical signal to build up in the nerve. When this electrical signal is big enough, it passes along the nerve to the brain, where the signal is interpreted as pain. It works by stopping the sodium entering the nerve ending at the site of the pain. This prevents an electrical signal building up and passing along the nerve fibres to the brain. In this way lignocaine causes numbness and relieves pain at the area it is applied to. Lidocaine is also the most important class 1B antiarrhythmic drug, use in refractory cases of status epilepticus. Inhaled lidocaine can be used as an antitussive , Lidocaine has also proved effective in treating jellyfish stings, both numbing the affected area and preventing further nematocyst discharge. In this study transcranial route is use to deliver drug to brain.

Apart from the established routes of drug administration practiced in modern medicine, *Ayurvedic* system of medicine uses a special route which involves oil therapies to the head. These therapies are used for centuries to treat diseases of the central nervous system [1]. The emissary veins draining blood from extracranial sites into the intracranial sinuses pierce a series of foramina present in the cranial bones. Scalp veins communicate with the sinuses of the brain via emissary veins. There are thirteen emissary veins connecting extracranial sites of the head with intracranial sinuses [2].TCR describes the passage of an oil solubilized drug moiety across the skin of the scalp including appendages of the skin such as sebaceous glands, walls of the hair follicles and sweat glands, through the cranial bones along with the diploe, the cranial bone sutures, the meninges and specifically through the emissary veins into the brain. It has to be noted that the total surface area of the skin along with the skin appendages adds up to a much larger area against the immediately visible plain skin surface in the scalp where the hair follicle density is very high compared to the rest of the skin.

The initial vigorous 'rubbing in' of the medicated oil is an essential part of the transcranial drug administration in order to bring the oil into intimate contact with the epithelium of the skin appendages. The effective catchment area for the proposed transcranial route is considered to be the area of the head lying above the contour drawn through the four points consisting of two corners of the mouth and the two ears in the case of all higher animals [3, 4]. The oil therapies of *Ayurveda* using the head include *Shirodara, Shiroabyanga, Shiropitchu, Shirovasthi* and *Shiropralepa* in which drugs are delivered by the transcranial route [5]. Propose study to evaluate Lignocaine gel as amnesic agent because Researchers are currently experimenting with drug-induced amnesia as a treatment for psychiatric disorders, such as post-traumatic stress disorder and memory related disorders, such as dementia and Alzheimer's disease. By understanding the ways in which amnesia-inducing drugs interact with the brain, researchers hope to better understand the ways in which neurotransmitters aid in the formation of memory. By stimulating rather than depressing these neurotransmitters, memory may improve.[6]

II. MATERIALS AND METHODS

2.1 Experimental Animals:

Adult albino wistar strain rats (100 ±20 Gms) and albino mice (20±6) of either sex were procured and were grouped randomly. The rats were acclimatized for one week in the animal house facility. They were housed in polypropylene cages at an ambient temperature of 25±1°C with a natural dark-light cycle. They had free access to standard pellet diet and water given *ad libitum.* All experiments were conducted in the day time (9:30 AM to 5:00 PM). The study was approved by institutional ethics committee (CPCSEA registration no. -1156/ac/07/CPCSEA).

2.2 Drugs/ Chemicals:

[1] Standard drug Scopolamine
[2] Water for Injection
[3] Lignocaine gel 2%
[4] Seasm oil

2.3 Laboratory animals for transcranial drug delivery experiments:

All the groups received the vehicle, standard drug and the test drug one hour prior to each experiment. Animals were selected and divided into groups (n=6). It was studied for Rod Walking Test (RW), Locomotor Activity Test (LA)**,** Elevated Plus Maze test (EPM)**,** Morris Water Maze Test (MWM)**,** Pole Climbing Test (PCT)**,** Pole Climbing Test on Trained rats (PCT-T). The animals are divided into four groups:

- Control WFI IP/ Saline
- Control TCR sesame oil
- Standard Diazepam (IP)
- Test group Lignocain TCR

2.4 Method of transcranial drug administration:

The hair of the scalp of animal was trimmed without injuring the skin for the transcranial application. The fourth group consider as a test group was treated with 2% lignocain by applying on to the hair trimmed bald area of the scalp followed by 'rubbing in' for 1 min with gentle massage by which it was to facilitate the oil solution come into contact with the skin and its appendages of the scalp properly. Similarly second group consider as a control applied sesame oil transcranially.

2.5 Experimental Procedure:

2.5.1 ***Rod Walking Test*****:** The ability of mice to balance on a stationary, horizontal rod and walk on it to come in at one end of the rod measures cognitive study and learning activity. Animals were placed in the center of a rod (100 cm long, 5 mm in diameter, and positioned 23 cm above the table surface), parallel to it, and their latency to transfer to its one end is recorded. All mice are trained for five days, and then tested for transfer latency for all the group of animals.

2.5.2 ***Locomotor Activity Test:*** Locomotor activity is easily measured using actophotometer which operates on photoelectric cells connected with a counter. When a beam of light falling on the photocell is cut off by the animal a count is recorded and displayed digitally. Each rat was placed individually in the activity cage floor for 05 min. The animals were placed in the actophotometer for recording the activity score at 0, 30, 60 and 90 minutes of drug administration

2.5.3 ***Elevated Plus Maze test*****:** The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in rat. The apparatus consisted of two open arms (50 cm × 10 cm) and two covered arms (50 cm × 40 cm ×10 cm). The arms extended from a central platform (10cm×10cm) and the maze was elevated to a height of 50 cm from the floor. On the first the day, each rat was placed at the end of open arm, facing away from central platform. With little modification transfer latency (TL) was taken as the time taken by the rat to move into any one of the covered arms enter with all its four legs where opposite gender of rat are placed in any one of the covered place to observe retention memory of test rat to come faster toward that area. TL was recorded on the first day for the each animal. The rat was allowed to explore the maze for another 2 min and returned to its home cage. Retention of this learned task was examined 24 h after the first day trial [7, 8].

2.5.4 ***Water Maze Test*****:** The Morris water maze consisted large circular pool, 1.50 m across and 0.60 m high filled with water, which was made opaque by adding milk. Water provided a uniform intramaze environment, thus eliminating any olfactory interference. A 28x10 cm rectangular escape platform was constructed of water resistant material and covered with material that allows the animal to remain on top when it is submerged. The platform was 28 cm in height so that it could be submerged 2 cm below the level of water surface. The water temperature was maintained at 26 ±2 °C. The animals were given a daily session of three trials per day. Latency time to reach the platform was recorded in each trial. Significant decrease in latency times from that of the first session was considered as successful learning, whereas an increase in latency times consider as amnesia to animal [9]. With little modification at first all rats has been trained for all six days and on last seventh day only has been administered diazepam to see the difference in transfer latency among different group.

2.5.5 ***Pole Climbing Test*****:** [10, 11]

Cook's Pole Climbing Apparatus use to study cognitive function, mainly a response to conditioned stimuli during learning & its retention. The apparatus has an experimental chamber (25 × 25 × 25 cm) with the floor grid in a soundproof enclosure. Scrambled shock (6mA) is delivered to the grid floor of the chamber composed of stainless steel rods. A pole, 2.5 cm in diameter, hangs inside the chamber through a hole in the upper center of the chamber. The study rat was placed in the chamber and allowed to explore the chamber for 45 seconds. Conditioned stimulus (CS) i.e buzzer signal was turned on and unconditioned stimulus (US) i.e electric shock delivered through grid floor for 45 Sec. Animal learned to associate the buzzer with the impending foot shock and was capable of avoiding the foot shock by climbing the pole after buzzer signal. Avoidance response was defined as climbing reaction time <10 Sec only; and escape response was climbing after applying reaction time >10 Sec. Every rat was subjected to maximum 05 trials on 1st day, and 24 hrs later, rat was subjected to Relearning trials (2nd day 3 trials and on 3rd day one trial) and transfer latency was noted to check the retention of Conditioned Avoidance Response (CAR) and escape response. Animals were screened by using this model and those who demonstrated at least one escape response either on day one or two were included in the study.

2.5.6 ***Pole Climbing Test on Trained rat*****:** By this method we studied anterograde amnesia of Diazepam on trained rat in Pole climbing apparatus. Every rat was subjected to maximum 05 trials on each day to responding Conditioned Avoidance Response (CAR) and perfect for it if the response is <2 seconds. Animals were screened by using this model and those were <2 seconds included in the study.

2.6 Statistical Analysis: All results were expressed as mean ± standard error of mean (S.E.M.). Data was analyzed using one way ANOVA and two way repeated measures followed by Tukey's multiple comparisons and student's unpaired t test using GraphPad Prism statistical software. $P < 0.001$ was considered as statistically extremely significant.

III. RESULTS AND DISCUSSIONS

3.1 Rod Walking Test: The Test group Lignocain TCR revealed a statistically significant increase in transfer latency in rod walking as compared to the control animals receiving only the vehicle and sesame oil (TCR) as like in Standard Diazepam treated group IP. The results were shown in table 2 and bar diagram with statistical significance value in Fig: 1

Table: 2 Effect of transcranial Lignocain and IP diazepam on rod walking test: Values are mean ± SEM of 6 animals per group;

TREATMENT GROUP	ESCAPE LATENCY IN SECONDS	
	DAY 1	DAY 2
Control WFI IP/ Saline	9.67 ± 0.558	11.5 ± 0.67
Control TCR sesame oil	10.83 ± 0.703	11.167 ± 0.543
Standard Diazepam (IP)	8.83 ± 0.543	30.33 ± 1.978
Test group Lignocain TCR	8.50 ± 0.764	45 ± 4.539

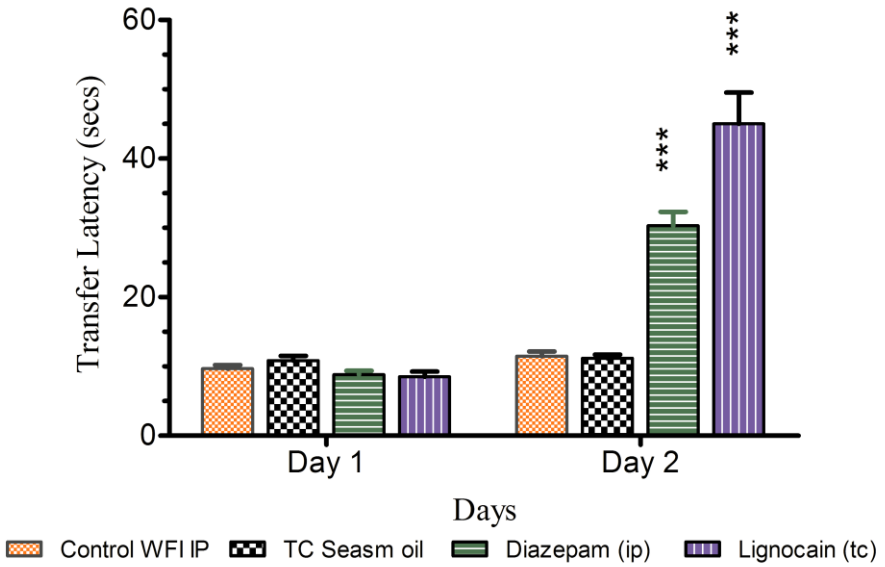

Fig 1: Bar Graph of mean transfer latency of mice in secs using rod walking metthod ; Statistical significance testing was done by one ways ANOVA and two way repeated measures followed by Tukey's multiple comparison test (n=6); ***P<0. 001 vs control (Saline and TCR); Test group Lignocain TCR and Diazepam (ip) standard, both are extreemly significant on day2.

3.2 Locomotor Activity Test: The Test group Lignocain TCR revealed a statistically significant reduction in locomotor activity as compared to the control animals receiving only the vehicle and sesame oil (TCR) as like in Standard Diazepam treated group IP. The results were shown in table 3 and bar diagram with statistical significance value in Fig: 2

Table: 3 Effect of transcranial Lignocain and IP diazepam on locomotor activity test: Values are mean ± SEM of 6 animals per group;

TREATMENT GROUP	LOCOMOTOR ACTIVITY (SCORES) in 5 mins			
	BEFORE TREATMENT 0	AFTER TREATMENT		
		30	60	90
Control WFI IP/ Saline	321.167 ± 4.92	322.167 ± 4.11	328.5 ± 5.228	328.5 ± 6.125
Control TCR sesame oil	347.167 ± 3.04	337.33 ± 2.15	345.66 ± 4.638	338.16 ± 4.87
Standard Diazepam (IP)	361.83 ± 4.36	226.33 ± 6.32	187.66 ± 8.4	173.66 ± 5.1
Test group Lignocain TCR	310.167 ± 5.7	272.5 ± 5.88	189.83 ± 4.38	133.8 ± 6.35

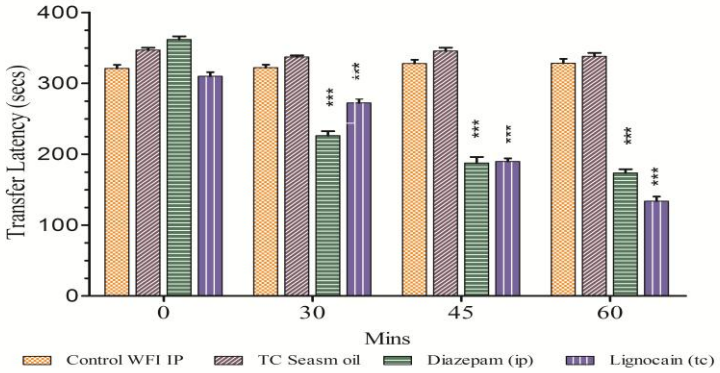

Fig 2: Bar Graph of mean Locomotion action for 05 mins in mice; Statistical significance test was done by t test following one way ANOVA and two way repeated measures as per Tukey's multiple comparison test (n=6); *** denotes P<0.001 vs control (Saline and TCR); Test group Lignocain TCR and Diazepam (ip) standard, both are highly significant.

3.3 Effect of Transfer Latency using Elevated Plus Maze: Transfer latency was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs. With little modification an opposite gender of rat is placed in any one of the covered place to observe retentive memory of test rat to come faster toward that area. Significant increase in TL value of retention indicated loss in memory. Lignocain TC (2%) showed increase in TL of the second day (after treatment) in rat ($p<0.01$) when compared to respective control groups (IP and TC) indicating significant memory loss (Fig. 3). Results of mean±SEM were shown in table 4 and bar diagram with statistical significance value presented in Fig: 3

Table 4: Effect of transcranial Lignocain and IP diazepam on elevated plus maze test: Values are mean ± SEM of 6 animals per group;

TREATMENT GROUP	TRANSFER LATENCY (SECONDS)	
	BEFORE TREATMENT	AFTER TREATMENT
Control WFI IP/ Saline	25.167 ± 0.946	24.83 ± 1.35
Control TCR sesame oil	24.167 ± 1.922	25.833 ± 1.108
Standard Diazepam (IP)	23.167 ± 1.778	46.83 ± 2.28
Test group Lignocain TCR	12.5 ± 0.992	35.5 ± 6.402

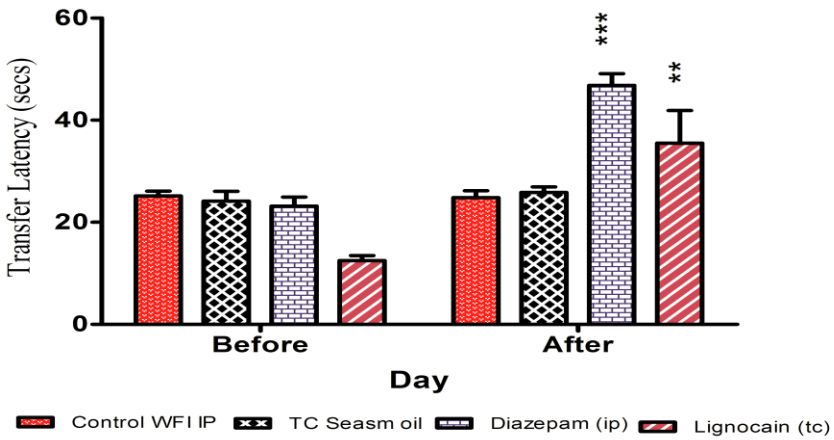

Fig. 3: Bar Graph of mean Transfer Latency in elevated plus maze using rat. Statistical significance test was analyzed by one way ANOVA and two way repeated measures followed by Tukey's multiple comparison test (n=6) and students unpaired t Test; *** denotes P values <0. 001 was considered as statistically significant vs control (Saline IP and TC); Standard Diazepam (IP) and Test group Lignocain TC highly significant for its amnesic effect.

3.4 Water Maze Test: Evaluation of escape latency

Results indicated that all animals of different group showed almost same transfer latency during training period, but in transcranially applied test group Lignocain TC (4mg/kg) showed a significant increase in transfer latency on the second phase, day 7 in rat ($p<0.001$) when compared to respective control groups (IP and TC) indicating significant memory loss (Fig. 4). The lignocain transcranial route shown an effect of memory deficit similar to IP administration of diazepam (4mg /ml) and it was found to be significant ($p<0.01$). Results of mean±SEM were shown in table 5 and bar diagram with statistical significance value presented in Fig: 4

Table 5: Effect of transcranial Lignocain and IP diazepam on water maze test: Values are mean ± SEM of 6 animals per group;

TREATMENT GROUP	TRANSFER LATENCY (SECONDS)		
	DAY 3	DAY 5	DAY 7
Control WFI IP/ Saline	30 ± 1.48	22.167 ± 0.654	16.667 ± 0.882
Control TCR sesame oil	27 ± 1.155	20.833 ± 1.014	12.5 ± 1.147
Standard Diazepam (IP)	23.5 ± 1.803	17.83 ± 2.469	38 ± 6.83
Test group Lignocain TCR	24.5 ± 1.979	19.33 ± 1.892	43.66 ± 7.135

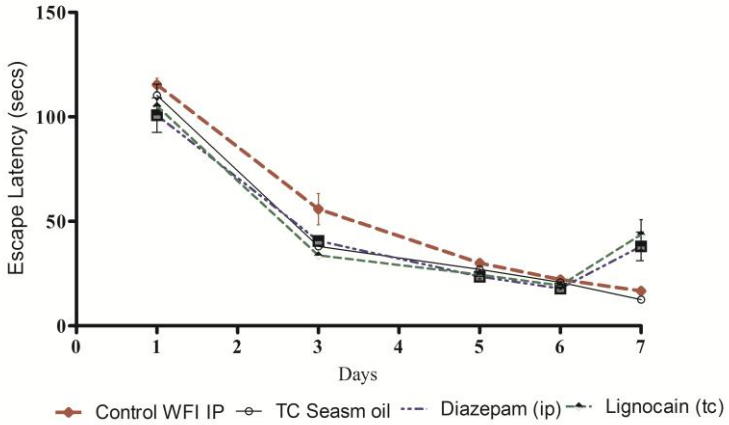

Fig 4: The transfer latency of transcranial Lignocain and IP diazepam of rat in secs using MWM ; Statistical significance test was analyzed by one way ANOVA and two way repeated measures followed by Tukey's multiple comparison test (n=6) and students unpaired t Test; *** denotes P values <0. 001 were considered as statistically significant vs control (Saline IP and TC); Test group Lignocain TC highly significant on day7 when studied for its amnesic effect.

3.5 Pole Climbing Test: The Test group Lignocain TCR revealed a statistically significant increase in transfer latency in pole climbing test as compared to the control animals receiving only the vehicle and sesame oil (TCR) as like in Standard Diazepam treated group IP. The results were shown in table 6 and bar diagram with statistical significance value presented in Fig: 5

Table 6: Effect of transcranial Lignocain and IP diazepam on pole climbing test: Values are mean ± SEM of 6 animals per group;

TREATMENT GROUP	AVOIDANCE/ ESCAPE LATENCY (SECONDS) [Mean ± SEM]		
	DAY 1	DAY 2	DAY 3
Control WFI IP/ Saline	37.5 ± 1.708	18.667 ± 1.706	7.5 ± 1.176
Control TCR sesame oil	37.67 ± 1.054	17.16 ± 0.87	7.3 ± 0.803
Standard Diazepam (IP)	38.33 ± 1.085	35.16 ± 0.401	27.33 ± 1.002
Test group Lignocain TCR	40 ± 0.0	36.67 ± 0.715	11.66 ± 1.054

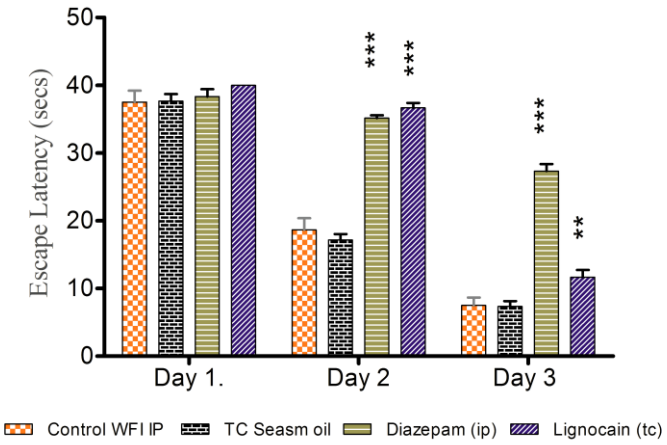

Fig 5: Bar Graph of escape latency of rat in secs using Pole Climbing Apparatus; Statistical significance testing was analyzed by one way ANOVA and two way repeated measures followed by Tukey's multiple comparison test (n=6) and students unpaired t Test; *** denotes P values <0. 001 statistically highly significant vs control (Saline IP and TC).

3.6 Pole Climbing Test on trained animal: The Test group Lignocain TCR revealed a statistically significant increase in transfer latency in rod walking as compared to the control animals receiving only the vehicle and sesame oil (TCR) as like in Standard Diazepam treated group ip. The results were shown in table 7 and bar diagram with statistical significance value presented in Fig: 6

Table 7: Effect of transcranial Lignocain and IP diazepam on pole climbing test on trained animal: Values are mean ± SEM of 6 animals per group;

TREATMENT GROUP	AVOIDANCE/ ESCAPE LATENCY (SECONDS)	
	0 mins	60 mins
Control WFI IP/ Saline	2.167 ± 0.167	2.167 ± 0.307
Control TCR sesame oil	2.50 ± 0.224	2.33 ± 0.211
Standard Diazepam (IP)	1.833 ± 0.307	8.83 ± 1.014
Test group Lignocain TCR	2.758 ± 0.896	7.417 ± 1.541

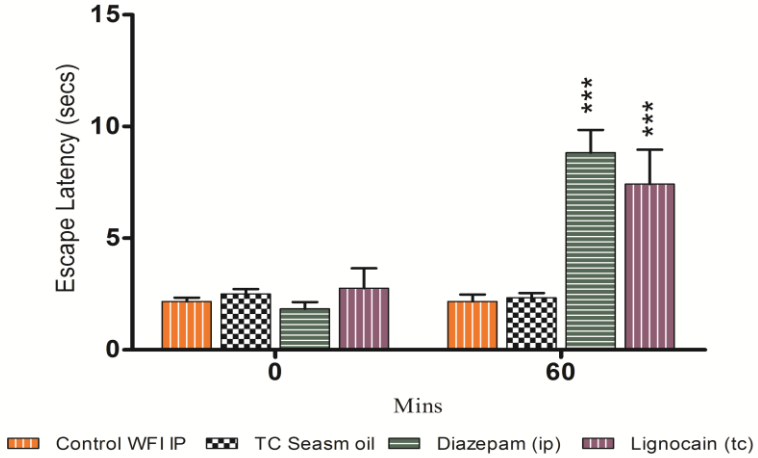

Fig 6: Bar Graph of escape latency of trained rat using Pole Climbing Apparatus; Statistical significance test was analyzed by one way and two way repeated measures ANOVA followed by Tukey's multiple comparison test (n=6) and students unpaired t Test; ***denotes P<0. 001 vs control (Saline IP and TC); Test group Lignocain TC and Diazepam (IP) standard both are highly significant at 60 min after treatment.

IV. DISCUSSION

Benzodiazepine drugs, such as midazolam, flunitrazepam, lorazepam, temazepam, nitrazepam, triazolam and nimetazepam are known to have powerful amnesic effects. In the study diazepam (4 mg/kg intraperitoneally) used as standard to evaluate an amnesic effect.The oil therapies of *Ayurveda* using the head include *Shirodara*, *Shiroabyanga*, *Shiropitchu*, *Shirovasthi* and *Shiropralepa* in which drugs are delivered by the transcranial route [5]. Trans Cranial Routes means drugs are delivered to the brain through transcranial route, it was stated that the passage of an oil solubilized drug moiety across the skin of the scalp, including appendages of the skin such as sebaceous glands, the walls of the hair follicles and sweat glands, through the cranial bones along with the diploe, the cranial bone sutures, the meninges and specifically through the emissary veins into the brain. The emissary veins draining blood from extracranial sites into the intracranial sinuses pierce a series of foramina present in the cranial bones. Scalp veins communicate with the sinuses of the brain via emissary veins. There are thirteen emissary veins connecting extracranial sites of the head with intracranial sinuses [2].

Many experimental models are currently available for the evaluation of agents that affect learning and memory process. Morris Water Maze is a traditional tool in assessing learning and memory performance in laboratory animals. Originally designed to evaluate the antianxiety agents, elevated plus maze has also been recently extended to measure the spatial long-term memory in animals [12, 13]. Passive avoidance behavior is used to examine the long term memory based on negative reinforcement [14]. In the present study, Standard Diazepam (IP) injected to induce significantly amnesia in normal rats tested in different models which are used in evaluation of learning and memory and are compareing with transcranially applied 2% Lignocaine gel in forehead of animals for this effect.

The results indicate that Lignocain on transcranial application shown effect like intraperitonial administration of diazepam in learning and memory model for memory loss. In Rod walking test the effect of transfer latency showed the test group Lignocain TCR statistically significant ($p < 0.001$) compared to the control animals receiving only the vehicle and sesame oil (TCR) as like in Standard Diazepam treated group IP. In Locomotor activity study the test group Lignocain TCR revealed a statistically significant ($p < 0.001$) reduction in locomotor activity as compared to the control animals receiving only the vehicle and sesame oil (TCR) as like in Standard Diazepam treated group IP. In an Elevated plus maze test Lignocain TCR (2%) showed increase in TL of the second day (after treatment) in rat ($p<0.001$) when compared to respective control groups (IP and TC) indicating significant memory loss. In MWM test observed that all animals of different group showed almost same transfer latency during training period, but in transcranially applied test group Lignocain TCR (2%) showed a significant increase in transfer latency on the second phase, day 7 in rat ($p<0.001$) when compared to respective control groups (IP and TC) indicating significant memory loss. The Test group Lignocain TCR revealed a statistically significant ($p<0.001$) increase in transfer latency in pole climbing test as compared to the control animals receiving only the vehicle and sesame oil (TCR) as like in Standard Diazepam treated group IP in a passive avoidance test evaluated using Pole climbing apparatus. Finally in another test done with little modification Pole climbing test on trained animal observed that the test group Lignocain TCR revealed a statistically significant ($p < 0.001$) increase in transfer latency in rod walking as compared to the control animals receiving only the vehicle and sesame oil (TCR) as like in Standard Diazepam treated group ip.In conclusion, based on the findings of the present study Lignocain TC application is effective in producing amnesia when it evaluated on different learning and memory evaluation model. This can be used as amnesic inducing agent upon transcranial application to the rodent.

V. ACKNOWLEDGMENT

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