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AMNESIC POTENTIALITY OF DIAZEPAM AT LOW DOSE ON ADMINISTRATION VIA TRANSCRANIAL ROUTE IN RODENTS

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ABSTRACT: The objective of study to evaluate a drug Diazepam applied transcranially to brain targeted drug delivery to screening for its amnesic effects, in different learning and memory paradigms viz., rod walking test, locomotor activity test, elevated plus maze test, water maze test, pole climbing test and pole climbing test on trained animal. Diazepam 4mg/ kg intraperitoneal used as a standard for comparing the test drug diazepam 4mg/ kg applied transcranially, whereas both water for injection intraperitoneally and seasme oil transcranially used as a control group. In rod walking test and locomotor activity test used mice for evaluating transfer latency and mean locomotion for 05 minutes and it was observed that Diazepam transcranially has a significant amnesic effect ($p<0.0001$) as compared to control, and as same like standard. In elevated plus maze, morris water maze, and the pole climbing test have been used rats for evaluating transfer latency, escape latency and avoidance latency; it was observed that Diazepam transcranial has a significant amnesic effect ($p<0.0001$) as compared to control, and as same like standard. In conclusion, based on the findings of the present study Diazepam transcranial 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.

INTRODUCTION: Diazepam produce *anterograde amnesia*, means "lose 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". The drugs are effective if it's delivered to the site of action, In the *Ayurvedic system* of medicine practices oil therapies to the head to treat diseases of the central nervous system [1].

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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, 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, 3]. The oil therapies of *Ayurveda* using the head include *Shirodara*,

Shiroabyanga, *Shiropitchu*, *Shirovasthi* and *Shiropralepa* in which drugs are delivered by the transcranial route [4]. An earlier study on transcranial route carried out with diazepam in sesame oil in which the centrally mediated muscle relaxant effect was studied by rotarod measurements has yielded positive results [5]. In another study Evaluation of effective medhya formulation on transcranial treatment on rat using different animal model uses to evaluate learning and memory and found to be effective in trancranial delivery [6]. In the present study Diazepam applied transcranially to brain targeted drug delivery to screening for its amnesic effects.

MATERIALS AND METHODS:

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

Drugs/ Chemicals:

- Diazepam 4mg/ml
- Sesame oil
- Water for Injection

Treatment groups:

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 rat (PCT-T). Group classifications are shown in a **Table1**.

TABLE: 1

NAME OF TEST	ANIMAL USE	GROUP			
		CONTROL WFI (IP)	CONTROL SESAME OIL (TCR)	STANDARD DIAZEPAM (IP)	TEST GROUP DIAZEPAM (TCR)
R W	Albino Mice	1	2	3	4
LA	Albino Mice	5	6	7	8
EPM	Rat (Wistar)	9	10	11	12
MWM	Rat (Wistar)	13	14	15	16
PCT	Rat (Wistar)	17	18	19	20
PCT (T)	Rat (Wistar)	21	22	23	24

Method of transcranial drug administration:

The hair of the scalp of mice in groups 2, 4, 6 and 8 was trimmed without injuring the skin. Each mouse in these four groups was treated with 0.1 ml of the respective oil application, in group 2 and 6 applied sesame oil as control and another two group 4 and 8 applied diazepam by applying drop wise on to the hair trimmed bald area of the scalp.

After the application was followed by 'rubbing in' for 1 min with gentle massage. This was to facilitate the oil solution come into contact with the skin and its appendages of the scalp properly. Group 1and 5 as a control (IP WFI) and group 3 and 7 (IP diazepam) as standard; sixty minutes after the application animals were subjected to various tests, like first locomotor activity test using photoactometer and next rod walking test.Similarly Wistar strain albino rat, groups 10, 14, 18 and 22, were treated with sesame oil and 0.2 ml diazepam oil preparation transcranially applied to group 12, 16, 20 and 24. Groups 9, 13. 17, 21 as a control (IP WFI) and group 11,15, 19 and 23 as standard (IP diazepam) tested for elevated plus maze, water maze test, pole climbing apparatus and test for trained animal on pole climbing apparatus.

Experimental Procedure:

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, then tested for transfer latency on group 1 (Control: WFI -IP) group 2 (Control: sesame oil TCR), group 3 (standard) Diazepam 2 m/kg, IP and group 4 as a test group (Diazepam TCR).

Locomotor Activity Test: Locomotor activity (horizontal activity) was measured using actophotometer mice were divided into four groups consisting of 6 per group. Two groups received Diazepam in different route; one is intraperitonially (group 7) and another one transcranially (group: 8).

The other two groups received control vehicle one group is taking WFI IP (group: 5) and another group is applied sesame oil transcranially (group: 6). Diazepam 2 mg/kg, i.p considers as a standard group. 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[7].

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 [8]. The group 9, 10, 11, and 12 were used as control (IP WFI), control (TCR sesame oil), standard (diazepam IP) and test (TCR diazepam) respectively for EPM test.

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].

The group 13, 14, 15, and 16 were used as control (IP WFI), control (TCR sesame oil), standard (diazepam IP) and test (TCR diazepam) respectively for MWM test. 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.

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. Cognitive function was assessed before and after drug treatment group 17, 18, 19, and 20 were used as control (IP WFI), control (TCR sesame oil), standard (diazepam IP) and test (TCR diazepam) test in the respective groups for PCT.

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. Cognitive function was assessed before and after drug treatment in the group 21, 22, 23, and 24 were used as control (IP WFI), control (TCR sesame oil), standard (diazepam IP) and test (TCR diazepam) in the respective groups for PCT on trained rats.

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 Graph Pad Prism statistical software**.** P < 0.001 was considered as statistically significant.

RESULTS AND DISCUSSIONS:

Rod Walking Test: The Test group Diazepam 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 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	10.67 ± 1.009	12.5 ± 1.009
Control TCR sesame oil	10 ± 1.009	11.5 ± 1.009
Standard Diazepam (IP)	8.5 ± 1.009	31.5 ± 1.009
Test group Diazepam TCR	9.5 ± 1.009	38.67 ± 1.009

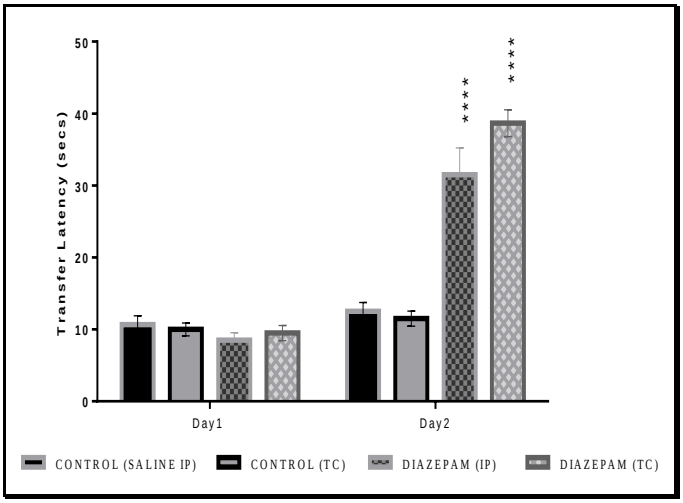

FIG 1: BAR GRAPH OF MEAN TRANSFER LATENCY OF MICE IN SECS USING ROD WALKING METHOD ; STATISTICAL SIGNIFICANCE TESTING WAS DONE BY ONE WAYS ANOVA AND TWO WAY REPEATED MEASURES FOLLOWED BY TUKEY'S MULTIPLE COMPARISON TEST (n=6)

****P<0. 0001 vs control (Saline and TCR); Test group Diazepam TCR and Diazepam (ip) standard, both are highly significant on day2.

Locomotor Activity Test:: The Test group Diazepam 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 AND IP DIAZEPAM ON LOCOMOTOR ACTIVITY TEST

TREATMENT GROUP	LOCOMOTOR ACTIVITY (SCORES) in 5 mins			
	BEFORE TREATMENT	AFTER TREATMENT		
	0	30	60	90
Control WFI IP/ Saline	313.8 ± 3.7	331.67 ±	334.5 ± 3.49	342.5 ± 2.97
Control TCR sesame oil	349.8 ± 1.4	334.67 ± 2.56	351.16 ± 2.7	346.16 ± 1.42
Standard Diazepam (IP)	372.6 ± 7.96	217.16 ± 8.61	192.83 ± 6.9	180 ± 5.01
Test group Diazepam TCR	332.8 ± 4.35	202.16 ± 4.57	179.0 ± 4.3	170.17 ± 5.41

Values are mean ± SEM of 6 animals per group;

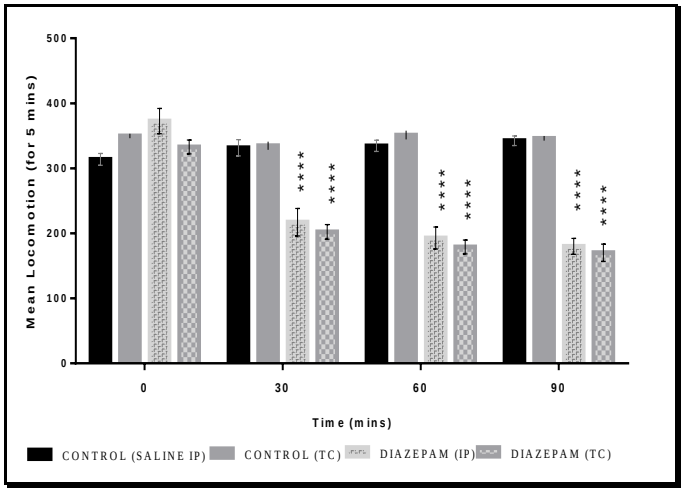

FIG 2: BAR GRAPH OF MEAN LOCOMOTION ACTION FOR 05 MINS IN MICE; STATISTICAL SIGNIFICANCE TEST WAS DONE BY T TEST FOLLOWING ONE WAY ANOVA AND TWO WAY REPEATED MEASURES AS PER TUKEY'S MULTIPLE COMPARISON TEST (n=6);

**** denotes P<0.001 vs control (Saline and TCR); Test group Diazepam TCR and Diazepam (ip) standard, both are highly significant.

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.

Diazepam TC (4mg/ml) showed increase in TL of the second day (after treatment) in rat (p<0.0001) when compared to respective control groups (IP and TC) indicating significant memory loss **(Fig. 3)**. The diazepam transcranial route shown an effect of memory deficit similar to IP administration of diazepam (4mg /ml) and it was found to be significant (p<0.0001). 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 AND IP DIAZEPAM ON ELEVATED PLUS MAZE TEST

TREATMENT GROUP	TRANSFER LATENCY	
	BEFORE	AFTER
Control WFI IP/ Saline	24.33 ± 1.145	24.5 ± 1.43
Control TCR sesame oil	22.83 ± 1.35	25.167 ± 0.98
Standard Diazepam (IP)	21.83 ± 1.85	48.83 ± 2.315
Test group Diazepam	22.83 ± 1.352	73.33 ± 3.49

Values are mean ± SEM of 6 animals per group

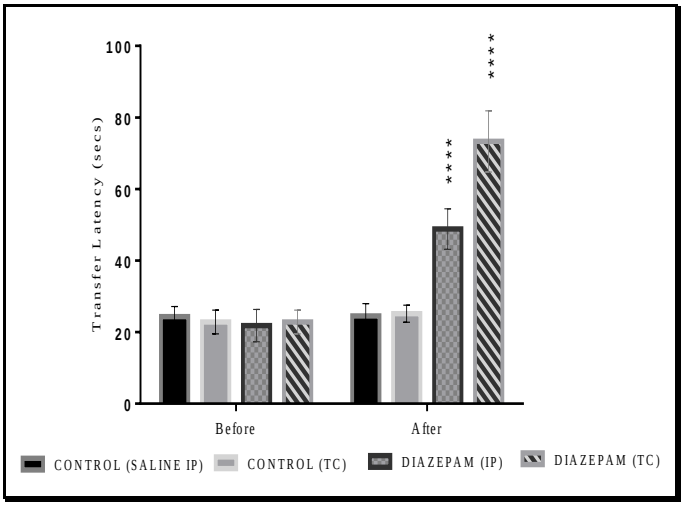

FIG. 3: BAR GRAPH OF MEAN TRANSFER LATENCY IN ELEVATED PLUS MAZE USING RAT. STATISTICAL SIGNIFICANCE TEST WAS ANALYZED BY ONE WAY ANOVA AND TWO WAY REPEATED MEASURES FOLLOWED BY TUKEY'S MULTIPLE COMPARISON TEST (N=6) AND STUDENTS UNPAIRED T TEST

**** denotes P values <0. 0001 were considered as statistically significant vs control (Saline IP and TC); Standard Diazepam (IP) and Test group Diazepam TC highly significant for its amnesic effect.

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 diazepam TC (4mg/kg) showed a significant

increase in transfer latency on the second phase, day 7 in rat ($p<0.0001$) when compared to respective control groups (IP and TC) indicating significant memory loss **(Fig. 4)**. 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 AND IP DIAZEPAM ON WATER MAZE TEST

TREATMENT GROUP	TRANSFER LATENCY (SECONDS)		
	DAY 3	DAY 5	DAY 7
Control WFI IP/ Saline	32.33 ± 2.32	22.66 ± 0.66	14.667 ± 0.615
Control TCR sesame oil	26.167 ± 0.60	20.167 ± 1.078	12.833 ± 0.91
Standard Diazepam (IP)	24.167 ± 2.08	19.66 ± 2.97	38.83 ± 6.85
Test group Diazepam TCR	25.667 ± 4.45	23.16 ± 3.628	37.33 ± 7.25

Values are mean ± SEM of 6 animals per group

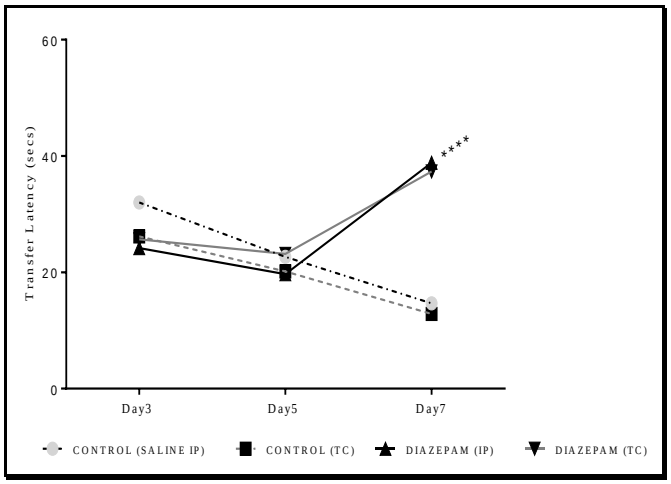

FIG 4: THE TRANSFER LATENCY OF TRANSCRANIAL AND IP DIAZEPAM OF RAT IN SECS USING MWM ; STATISTICAL SIGNIFICANCE TEST WAS ANALYZED BY ONE WAY ANOVA AND TWO WAY REPEATED MEASURES FOLLOWED BY TUKEY'S MULTIPLE COMPARISON TEST (n=6) AND STUDENTS UNPAIRED T TEST

**** denotes P values <0. 0001 were considered as statistically significant vs control (Saline IP and TC); Test group Diazepam TC highly significant on day7 when studied for its amnesic effect.

Pole Climbing Test: The Test group Diazepam 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 AND IP DIAZEPAM ON POLE CLIMBING TEST

TREATMENT GROUP	AVOIDANCE/ ESCAPE LATENCY (SECONDS)		
	DAY 1	DAY 2	DAY 3
Control WFI IP/ Saline	31.67 ± 0.989	13.33 ± 0.494	5 ± 0.577
Control TCR sesame oil	35.17 ± 0.833	15.33 ± 0.494	6 ± 0.365
Standard Diazepam (IP)	40 ± 0.0	35.5 ± 0.428	28.17 ± 0.792
Test group Diazepam TCR	39.17 ± 0.543	29.17± 0.703	20.83 ± 1.078

Values are mean ± SEM of 6 animals per group

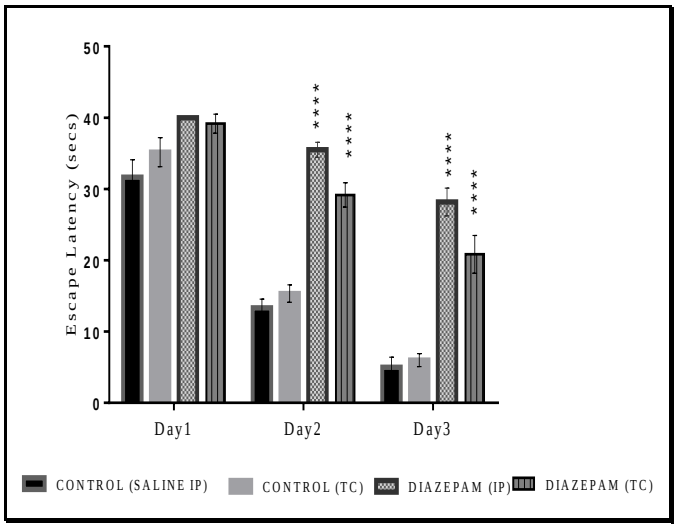

FIG 5: BAR GRAPH OF ESCAPE LATENCY OF RAT IN SECS USING POLE CLIMBING APPARATUS; STATISTICAL SIGNIFICANCE TESTING WAS ANALYZED BY ONE WAY ANOVA AND TWO WAY REPEATED MEASURES FOLLOWED BY TUKEY'S MULTIPLE COMPARISON TEST (n=6) AND STUDENTS UNPAIRED T TEST

**** denotes P values <0. 0001 statistically highly significant vs control (Saline IP and TC); Test group Diazepam TC and Diazepam (IP) standard, both are highly significant on day2 and day 3. **** P values < 0.0001 denotes Diazepam (TC) is highly significant as compared to Diazepam standard IP.

Pole Climbing Test on trained animal: The Test group Diazepam 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 AND IP DIAZEPAM ON POLE CLIMBING TEST ON TRAINED ANIMAL

TREATMENT GROUP	AVOIDANCE/ ESCAPE LATENCY (SECONDS) [Mean ± SEM]	
	0 mins	60 mins
Control WFI IP/ Saline	1.833 ± 0.307	1.333 ± 0.211
Control TCR sesame oil	2.0 ± 0.365	1.5 ± 0.224
Standard Diazepam (IP)	1.333 ± 0.211	9.33 ± 0.105
Test group Diazepam TCR	1.333 ± 0.211	8.833 ± 0.601

Values are mean ± SEM of 6 animals per group

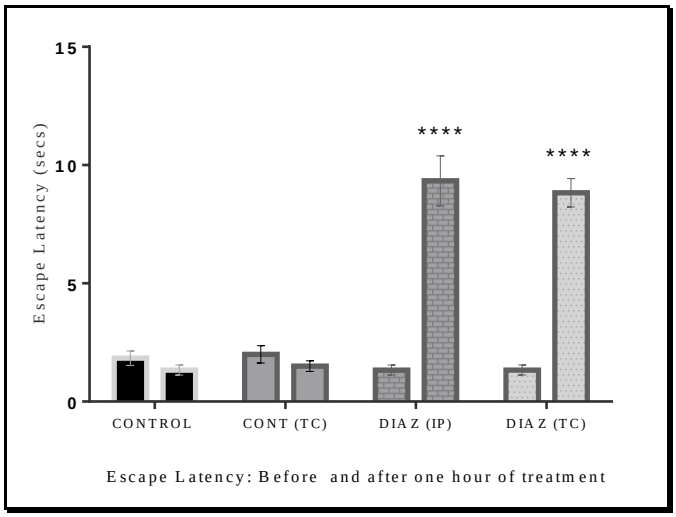

FIG 6: BAR GRAPH OF ESCAPE LATENCY OF TRAINED RAT USING POLE CLIMBING APPARATUS; STATISTICAL SIGNIFICANCE TEST WAS ANALYZED BY ONE WAY AND TWO WAY REPEATED MEASURES ANOVA FOLLOWED BY TUKEY'S MULTIPLE COMPARISON TEST (n=6) AND STUDENTS UNPAIRED T TEST

****denotes P<0. 0001 vs control (Saline IP and TC); Test group Diazepam TC and Diazepam (IP) standard both are highly significant at 60 min after treatment.

DISCUSSIONS: The oil therapies of *Ayurveda* using the head include *Shirodara, Shiroabyanga, Shiropitchu, Shirovasthi* and *Shiropralepa* in which drugs are delivered by the transcranial route [4]. Trains 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].

Diazepam produce *anterograde amnesia*, means "loss 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". Diazepam in the experimental study uses as an amnesic inducing agent. In the present study, Diazepam induced significantly amnesia in normal rats tested in different models which are used in evaluation of learning and memory and compare two different intraperitonial and transcranial routes of administration.

The results indicate that transcranial route is effective 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 Diazepam TCR statistically significant ($p < 0.0001$) 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 Diazepam TCR revealed a statistically significant ($p < 0.0001$) 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 Diazepam TC (4mg/ml) showed increase in TL of the second day (after treatment) in rat ($p<0.0001$) 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 diazepam TC (4mg/kg) showed a significant increase in transfer latency on the second phase, day 7 in rat ($p<0.0001$) when compared to respective control groups (IP and TC) indicating significant memory loss.

The Test group Diazepam 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 Diazepam TCR revealed a statistically significant ($p< 0.0001$) 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 Diazepam 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.

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