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Experimental evaluation of effect of Drakshasava on memory and learning of Wistar rats

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Abstract

Drakshasava is a traditional herbal preparation used in Ayurveda as a general tonic to treat various ailments. It primarily consists of draksha (grapes), along with multiple other herbal ingredients. During the manufacturing of the asava the alcohol is generated. There are claims about enhancement of the effect of Drakshasava by this self-generated alcohol. Present study was undertaken with the objective to evaluate the effects of Drakshasava, an Ayurvedic fermented formulation, on memory and learning in Wistar rats. Thirty-two Wistar rats were allocated into four groups: a control group, Drakshasava-LD & HD and standard drug treated. The Drakshasava-treated group received 2 ml/kg and 4ml/kg body weight and Piracetam was given to standard drug group orally for 21 days. Behavioral assessments were conducted using the Elevated Plus Maze and Morris Water Maze (MWM) to evaluate spatial learning, memory retention, and recall abilities. Rats treated with Drakshasava showed significant improvement in the EPM, Drakshasava-treated rats demonstrated improved retention and recall, evidenced by prolonged step-through latency. In the MWM test, no significant reduction in escape latency and time spent in the target quadrant compared to the control groups, indicating no effect on spatial learning and memory. Drakshasava enhances cognitive function moderately in Wistar rats, as evidenced by improved performance in behavioral tests. These findings support the potential use of Drakshasava as an antianxiety agent but not as a cognitive enhancer.

Kay words: Drakshasava, memory, learning, Wistar rats, Morris Water Maze, Elevated plus maze.

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Introduction

Drakshasava is a traditional Ayurvedic formulation made from fermented grape juice, along with various herbs and spices. Its general health benefits, including improving digestion, increasing appetite, and promoting vitality are well known. However, there has been growing interest in its potential cognitive-enhancing effects, particularly in the context of memory and brain function due to its antioxidant Properties, neuroprotection and stress reducing properties. While there are some studies that suggest the individual components of Drakshasava such as grapes and certain herbs may have cognitive benefits, but there is limited scientific research specifically on Drakshasava itself has been done. Most of the evidence comes from traditional use and preliminary studies [1].

Drakshasava contains draksha, kumara, dhatkipushpa, chitrak, kankol, chavak, ranuk, lavang, nagpushpa, trijat, marich, piper, jatiphal, pipalimoola sugar, etc. [2]. Asava is a special class of Ayurvedic formulation prepared by fermentation of fresh herbal juices [3] that contain self-generated alcohol during the process of preparation, which also serves as a preservative in the product [4,5] The alcohol produced through fermentation in Drakshasava can have both potential benefits and risks for memory and cognitive function. While moderate levels may enhance the bioavailability of beneficial compounds and potentially offer some cognitive benefits. It also facilitates rapid absorption of ingredients, acts swiftly, inhibits the growth of microorganisms and extends shelf life of the medication [6].

Previous preclinical studies have indicated that natural products rich in polyphenols, including grape-derived compounds, can improve memory and learning abilities in animal models. At the same time alcohol is a general CNS depressant and CNS depressant drugs affect learning and memory and produce subjective effects [7]. So it is assumed that it may affect short term and long term memory. Drakshasava contains alcohol generated during fermentation, however, there is limited scientific evidence specifically evaluating the cognitive effects of Drakshasava, particularly in the context of memory and learning in experimental animals models [8]. Hence the present study was undertaken to evaluate the effect of Drakshasava on memory and learning in wistar rats.

Materials and Methods

Study was started after obtaining the Institutional Animal Ethics Committee approval (BVDUMC/720/2023/002/007). Drakshasava was obtained from local Ayurvedic medicine shop. Piracitam and other required medicines were obtained from the chemist.

Animals used- 32 Wistar rats of either sex weighing 150-200g were used for evaluation. The animals were kept in the cages available in the laboratory in suitable environmental conditions as per the CCSEA guidelines. They were given free access to food and water from Aquaguard. Standard protocol was used for animal coding of animals.

Rats were divided randomly into 4 groups containing 8 rats each. Group 1: control- treated with saline, Group 2: Treatment with Drakshasava (2ml/kg body weight; this is the low dose extrapolated from the clinically used human dose), Group 3: Treatment with Drakshasava (4ml/kg body weight; high dose is the double the clinically used dose) & Group 4: Treatment with Piracetam (200 mg/kg, i.p.). Elevated Plus Maze and Morris Water Maze [9] was used to assess memory and learning in animals.

Elevated Plus Maze model- training was given for a period of 4 days, where each rat was placed at the end of an open arm not facing towards the central platform. Time taken by the rat with all its legs to move towards one of the closed arms or other open arm was recorded with the help of any maze recording system and that is known as the transfer latency. If the animal did not enter into one of the closed arms within 90s then he will be pushed into any of the 2 closed arms and the transfer latency was assigned as 90s during the training session. After 4 days of retention the task that the rat had learnt was examined. Drug treatment was given for a period of 21 days and the effect of Drakshasava on memory was evaluated.

Morris Water Maze Model [10] Each animal underwent daily trials at 5-minute interval in the water maze. During each trial, rats stood on the platform for 20 seconds. If a rat couldn't find the hidden platform within 120 seconds, rat was gently guided by hand towards the platform. The time taken by each rat to locate the hidden platform (escape latency) served as an index of acquisition over consecutive 4 days of trials. On the 5th day, the hidden platform was removed, and the time each animal spent searching in each quadrant and specifically in the target quadrant (Q4) was recorded. This time spent in the target quadrant (Q4) searching for the missing platform served as an index of memory retrieval.

Statistical Analysis:

One-way analysis of variance (ANOVA) used to compare the mean performance scores (e.g., latency to find the platform in the Morris water maze in across all treatment groups. Post-hoc analyses was done with Tukey's multiple comparison test to identify specific pairwise differences between treatment groups. Results are presented as mean ± SD.

Results:

Table 1: Effect of Drakshasava on time spent in open arm and closed arm on Elevated Plus Maze

Groups	**No. of Entries** in Open arm on day 21	**No. of Entries** in closed arm on day 21	**Time Spent** in open arm Pre test on day 0	**Time Spent** in closed arm Pre test on day 0	**Time Spent** in open arm Post test on day 21	**Time Spent** in closed arm Post test on day 21
Control	2.00±0.70	0.75±0.25	15.68±4.62	9.73±8.56	14.05±4.79	11.43±9.96

DKS LD	1.12±0.44	0.5±0.18	14.55±4.93	14.02±12.64	19.05±4.42	8.53±8.65
DKS HD	1.5±0.59	1.5±0.42	15.57±3.67	16.68±8.65	20.55±4.3	8.3±8.54
Piracetam	1.12±0.44	1.3±0.56	15.10±4.48	13.37±11.58	21.33±3.55	6.77±8.24

All values are expressed as mean ± SD (n=8); DKS-Drakshasava, LD-Low dose, HD- High dose, *p<0.05 when compared to control. One-way ANOVA, Dunnett's Multiple Comparison post hoc tests.

Number of entries in open arm in the low dose and Piracetam treated group was almost similar but with the high dose DKS number of entries in open arm were more than LD DKS & Piracetam.

Time spent in the open arm post treatment, Increase in the time spent in open arm was observed with the DKS LD, HD & Piracetam though it was not statistically significant. (Table 1)

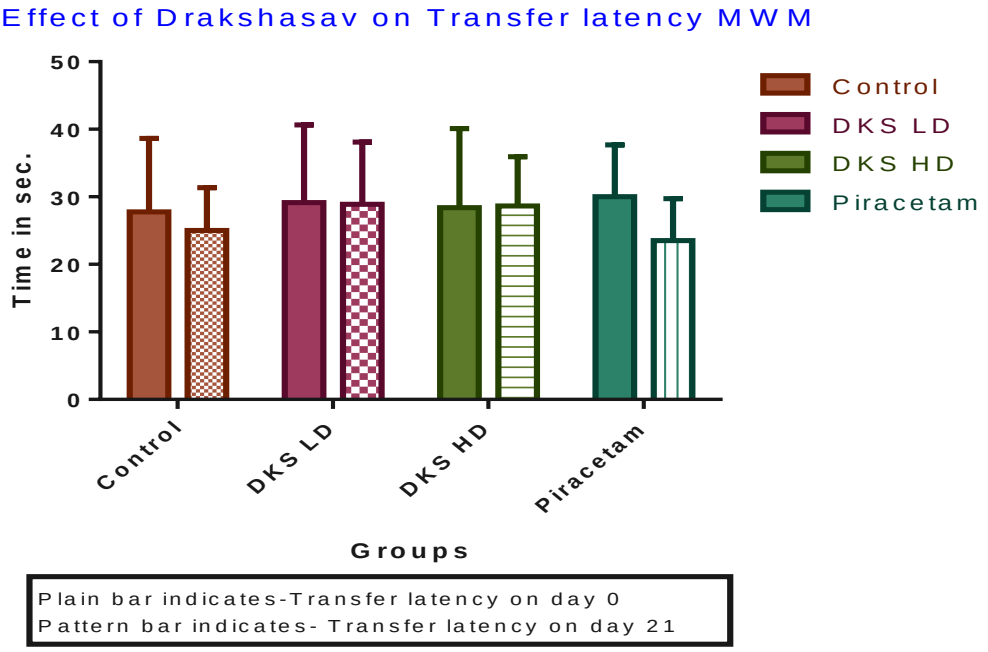

Figure 1: Effect of Drakshasava on transfer latency on Morris water maze

There was no significant difference in TL of DKS LD & HD treated animals when compared with the Control and standard drug Piracetam group.

Discussion:

Drakshasava is a traditional Ayurvedic medicine prepared from grapes has a long history of consumption as a health tonic due to its various purported benefits. The primary ingredient, grapes, is rich in antioxidants, vitamins, and minerals, which are believed to promote overall health. In Ayurveda, it's often recommended for improving digestion, boosting the immune system, and promoting general health benefits [11]. Additionally, it's sometimes used to treat conditions like anaemia and respiratory disorders [12]. Memory is the cognitive ability to encode, store, retain, and recall information and past experiences [13]. It's a crucial aspect of human cognition and plays a fundamental role in our daily lives, influencing everything from learning and decision-making to identity and personal narratives.

Alcohol can have significant effects on memory, both in the short term and over prolonged use [14]. Even moderate alcohol consumption can impair short-term memory [15]. The amount of alcohol required to cause memory loss varies from person to person and other factors like tolerance, body weight, and individual susceptibility to alcohols effect [16]. In some individuals memory impairment can occur even after consumption of small amount of alcohol. This can manifest as difficulty in remembering recent events, conversations, or

details encountered while under the influence of alcohol. Alcohol-induced blackouts involve periods of amnesia even with relatively low levels of alcohol consumption [17].

Our study investigated the effects of Drakshasava, an Ayurvedic medicine containing 5-10% self-generated alcohol [18] on memory and learning in rats after administration for 21 days. We employed the Morris Water Maze and Plus Maze tasks to assess these cognitive functions. Despite the lower number of entries into the open arm in all drug-treated groups compared to the control group, the difference was not statistically significant. This suggests that while there may be a trend towards reduced exploration of the open arm in control group than the drug treatment group, it did not reach a level of statistical significance. (Table 1) High dose Drakshasava appeared to be more effective than low dose Drakshasava and Piracetam in influencing behavior in the elevated plus maze. This indicates a dose-dependent response to Drakshasava, with higher doses potentially exerting stronger effects on anxiety-like behaviors. Results are similar as seen in our previous studies [2]. This suggests that drug treatment, particularly with high dose Drakshasava, led to a pronounced shift towards spending more time in the less anxiety-provoking open arm of the maze. This further supports the notion that drug treatment reduced anxiety-like behaviors, as animals spent less time in the more anxiety-provoking closed arm. The absence of freezing behavior in the animals suggests that they did not exhibit significant fear or stress responses during the experiment.

The Morris water maze is valued for its ability to assess spatial learning and memory in a relatively naturalistic setting and its translational relevance to human spatial cognition [19]. The findings indicate that DKS did not demonstrate any significant effects on memory and cognitive function compared to standard drug Piracetam [20] or control conditions, as measured by TL in the Morris water maze test. The lack of significant difference in TL between the DKS-treated group and the Piracetam group suggests that DKS did not outperform the effects of Piracetam, a known cognitive enhancer. (Figure 1) This is a significant finding because it suggests that DKS may not be as effective as Piracetam in improving cognitive function but at the same time there was no decrease in the performance compare to control suggest that alcohol generated in DKS did not affect the learning and memory. Drakshasava contains polyphenols and antioxidants, have been associated with cognitive benefits. Resveratrol is a natural phenolic substance found in the epidermis of grapes [21] and showed memory enhancing in patients of Alzheimer's and dementia. it may help protect against age-related cognitive decline and neurodegenerative diseases like Alzheimer's disease by its antioxidant and anti-inflammatory effects. However, in present study, DKS did not produce any significant impact of on memory or learning rather it has showed effects similar to the control and standard control group but reduced anxiety.

Conclusions:

This study investigated the effects of Drakshasava, an Ayurvedic medicine containing self-generated alcohol, on memory and learning in rats. We employed water maze and plus maze tasks to assess these cognitive abilities following 21 days of Drakshasava administration. Our findings revealed that Drakshasava treatment did not significantly impact memory or learning in rats compared to the control group. These results suggest that short-term administration of Drakshasava at the tested dose may be safe for cognitive function, reduces anxiety, but not showing significant improvement in memory and learning. However, further studies are warranted to explore the effects of chronic administration, higher dosages, and potential interactions with other medications.

Limitations:

This study was limited by the use of a relatively short treatment period (21 days). Long-term administration of Drakshasava might yield different results. Additionally, the study focused on a single dose; investigating the effects of a wider dosage range could provide more nuanced insights.

Conflict of Interest- The authors declare that there are not any potential conflicts of interest

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