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To Explore Nootropic Activity of *Artemisia roxburghiana* Ethanolic Leaf Extract on Wister Rats.

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KEYWORDS
Dementia, Piracetam, artemisia roxburghiana, scopolamine,Y-maze test

ABSTRACT:

Introduction:Progressive cognitive impairment is the hallmark of dementia, which invariably causes problems with all knowledge-based tasks.It often begins with an impairment in the ability to acquire new memories.An ethanolic extract from *Artemisia roxburghiana* (Asteraceae) leaves was tested for its effects about memory and learning using the Y-maze test, locometer activity, and brain histology.Rats were given the ethanolic leaf extract orally at two different dosages (100 mg/kg and 400 mg/kg) for seven days.

Objectives:To explore Nootropic activity of Ethanolic extract of *Artemisia roxburghiana* leaves on wistar rats.

Methods:In this study we used thirty female wister rats in five groups each group contains six rats animals were given doses of ethanolic leaf extract of *artemisia roxburghiana* 100mg/kg and 400mg/kg for seven days to test the nootropic activity by using Y- maze test and locometer activity.After that examined the histological dissection of rat's brain tissues.

Results:With the Y-maze task, an ethanolic extract of *Artemisia roxburghiana* demonstrated a greater percentage of spontaneous alteration and an altered behavior response.In locometer activity it is observed that the ethanolic leaf extract of *artemisia roxburghiana* showed nootropic activity by increasing locometer activity.In histological study, effective regeneration scores were shown using low and high extract doses compared to other groups.

Conclusions:The data suggests that the ethanolic leaf extract of *artemisia roxburghiana* demonstrated potential nootropic activity.

1. Introduction

Alzheimer's disease is often accompanied by dementia, an age-related mental illness (1).AD is a neurodegenerative, cerebrovascular illness that progresses over time(2).In AD the greater typical kind of senile dementia, memory loss coexists with impairment of the basal forebrain cortical neurons that are cholinergic (3).The loss of cholinergic neurons, aberrant tau phosphorylation, oxidative stress, and modified protein processing leading to aberrant β-amyloid peptide (Aβ) buildup are all part of the multifactorial etiology of AD.A relatively new class of psychoactive medications known as nootropics specifically enhances memory, learning, and cognition in the integrative function brain and spinal cord (4).The disintegration of the nervous system, with neurons as the primary target, is known as neurodegeneration.Neurodegeneration is a phrase that refers to a variety of disorders caused by the progressive loss of neuronal structure or uses(5).When discussing the causes of neurode generation, it's critical to emphasize disequilibrium in protein homeostasis, neuro inflammation, and mitochondrial failure(6).The term "smart drugs" is commonly used to describe nootropics.The Greek verb "tropein," which means to lead, and the noun "nöos," which means "thought," are combined to form the word "name."These substances fall under different categories. Whereas some authors distinguish between medications that increase brain metabolism and traditional nootropics, others combine the two or refer to nootropics as "cognitive effects"(7).Free radicals, which have been connected to diseases like cancer, heart disease, and others, can damage cells. The effects of various herbs on animals' cognitive function are currently the subject of several experimental investigations.The benefits of various

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herbs on cognitive function have been well-documented by these investigations, which have also contributed to the understanding of the methods behind these effects.A boost to mental abilities such as motivation, creativity, and recall.The Romanian chemist and psychologist who coined the term "nootropic" first used it to refer to a novel class of medications that he thought may enhance cognitive performance (8).Piracetam, aniracetam, modafinil, and caffeine are a few typical nootropic examples.To completely understand the effects and potential hazards of various nootropics, fresh study is necessary as the scientific data supporting their efficacy is sparse(9).Indian herbal remedies have been utilized to address cognitive decline, epilepsy, and sleeplessness (10).Numerous plants have been the subject of in-depth research and claimed to have memory-boosting qualities(11).Ayurvedic medicine makes extensive use of the plant artemisia roxburghiana.*Artemisia roxburghiana* plants are utilized for treating malarial fever and colds according to ethnobotany (12).The purpose of the study is the *artemisia roxburghiana* plant's nootropic activity, or memory-enhancing properties.

2.Objectives

To explore Nootropic activity of Ethanolic extract of *Artemisia roxburghiana* leaves on wistar rats by verification of botanical specimens and making an ethanolic *Artemisia roxburghiana* leaf extract and then evaluate the effect of *Artemisia roxburghiana* ethanolic extract in Scopolamine induced neuro toxicity in rats by measuring the following parameter

Behavioral – Locomotor activity, Y Maze test

Histopathology-Eosin and hematoxylin staining of brain cells.

3.Methods

Plant profile:

Kunja, or *Artemisia roxburghiana*, was the plant selected for the study.Formally known as *Artemisia roxburghiana*, kunja is a plant that is a member of the Asteraceae family, which includes sunflower plants(13).Cutting the stem of the artemisia plant open to reveal the petiole section revealed the following pharmacognostical characteristics: polygonal cells, xylem, phloem, cambium cell, upper and lower epidermis, and vascular bundles.Clasticulate trichomes and pluricellular covering trichomes are two of *Artemisia roxburghiana* leaves' most important features (14).South India is a commercial producer of davana oil, which is applied to cure fever as well as for get rid of intestinal worms(15).Aromatic and therapeutic plants include secondary metabolites, which are used in a number of industries including pharmaceuticals, cosmetics, and medicine(16).

Plant collection and identification:

This plant was discovered in Uttarakhand, India's Chamoli district, at a height of roughly 3,584 meters, or 10,000 to 12,000 feet, above sea level (18 meters)(17).The plant's identify was confirmed by the Botanical Survey of India, Dehradun. After being well cleansed beneath flowing water and then rinsed with using purified water, the aerial parts of *Artemisia roxburghiana* were left to dry for two weeks at room temperature in the shade. Following that, until additional research was conducted, the dried plant components were kept dry and free of humidity in tightly sealed containers(18).

Plant material extraction:

Using a mortar and pestle, the dried leaves of the plant were cleaned and broken into little pieces.After that, 100 milliliters of analytical-grade ethanol were used to completely extract ten grams of plant material over the period of four days (using a soxhlet apparatus).After that, the extract was submerged in a bath of water that was heated to between 60 and 70 degrees Celsius so it could evaporate. An initial phytochemical test was conducted on the extract using a standard screening protocol.

Drugs and chemicals:

The following medications and substances were employed: piracetam tablets (IKON Pharmachem PVT limited) and scopolamine hydrobromide (hysocine)-Sovereign Pharma Pvt.ltd. The remaining chemicals were all purchased from standard companies and were of the highest purity and analytical grade.The term "water" refers to double-distilled water, and an orogastric cannula was utilized to administer medications orally.

Animals:The tests used female wistar rats weighing between 200 and 250 grams.The Siddhartha Institute of Pharmacy in Dehradun, India's faculty of pharmacy's

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primary animal house housed the animals.Groups of rats were kept in the housing of five in controlled environments with a light and dark cycle in standard laboratory settings.They could have ordinary food and water without any restrictions.Approval was obtained to conduct this investigation from the ethical committee of the pharmacy faculty at the Siddhartha Institute of Pharmacy in Dehradun.

Experimental protocol:

Adult After being collected, female wistar rats (body weight 200–250g) aged 9–11 weeks will be kept in a 12-hour light, water, and food supply.To assess their answers on the Y maze test and locometer activity, thirty healthy Wistar rats of the female sex were chosen at random and split into five each grouping contains six animals.

An overview of the animal groups is given below:

Group I (Negative control): No treatment

Group II (Positive control): 1 mg/kg scopolamine S.C

Group III (Standard control): 500 mg/kg of piracetam oral route

Group IV: (Test A) :Ethanolic extract of *Artemisia roxburghiana* (100 mg/kg) oral route

Group V: (Test B) :Ethanolic extract of *Artemisia roxburghiana*(400 mg/kg) oral route

This experiment was conducted according to the standard methodology that CPCSEA,India advised. The study protocol(1435/PO/Re/S/11/CPCSEA 2011) was approved by the Siddhartha Institute of Pharmacy's IAEC's Ethical Committee in Dehradun, India.

Pharmacological activity:

Models for behavioral testing:

Figure 1: Y- maze apparatus

The Y-maze test:

The Y-shaped maze was the exteroceptive paradigm utilized to assess rats' acquisition of spatial memory.The device was made of basic wood and features three similar arms.Using spontaneous behavioral changes, the Y-maze challenge trains rats in spatial working memory. When food is offered as an incentive, animals are either compelled to follow a particular search pattern or make fewer mistakes when looking for food.Thus, the most important variables to be monitored for the assessment of medication effects post-training are temporal measurement and error scoring(19).The Y-maze is a wooden maze with three arms and 120 degree angles between the two arms.The dimensions of each arm are forty centimeters long, thirteen centimeters wide, and three cm high.The three identical arms have random designs: the reward arm(B),which includes food stimuli, the other arm (C),and the start arm(A), where the animal begins to investigate.For the initial two minutes, the number and order of manually inserted arms were noted.Rats typically enter the maze one at a time and examine each arm thoroughly(20).The alternations are indicated by the rats' arms entering.Arms were sprayed down with water to remove any odors and residue in between experiments.The rats' arms entering indicate the alternations.In between trials, arms were rinsed off with water to get rid of any leftovers and smells(21).The percentage alternation score for each animal was calculated by this equation:

% alternation :[(number of alternations) / (total arm entries - 2)] x 100

Experimental procedures:

All groups received the same dosage for seven days; Group III,VI, and V received scopolamine (1 mg/kg s.c.) thirty minutes after the final dose was administered on the seventh day, thereby inducing dementia.On the seventh day, Group II, the negative control group, received one dosage of 1 mg/kg s.c. of scopolamine. Trials in the Y maze were conducted thirty minutes later(22).

Locometer activity:

In rats and mice, locomotor activity has long been used to assess the impact or functionality of medication,genes,and disease models(23).Actophotometer was used to measure

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locometer action. Moreover, it may shock rats with up to 100 volts of electricity to elicit action(24).Within the actophotometer's test chamber, the rats' locomotor activity was recorded, which had dimensions of 289 measured in inches,eighteen inches in width, and twelve inches in height.Six light sources were placed on two of the test room's adjacent lateral walls: six at a higher level, two inches from the base, and one at a lower level, one inch from the base.The sensors on the other two walls were each positioned across from a light-receiving photocell-based receptor. Consequently, the total amount of light been evaluating the rats' movements.The device captured a record every time an animal cross the light.Animals were individually inserted into the activity meter for the locomotor activity trial, and the overall activity count was recorded for the whole 10-minute observation time(25).The activity's units are arbitrary and determined by beam blockages brought on by an animal's movement(26).

Figure 2:Actophotometer cage

Experimental procedure:

Every rat was separately inserted into an actophotometer for ten minutes one hour after treatment, during which time its locomotor activity was assessed.Every group's variation in locomotor activity was noted(27,28).For 7 days group I (negative control) was not administered any treatment.Scopolamine(1mg/kg S.C)were administered on 7 th day to group II positive control.piracetam (500mg/kg orally) administered to group III standard treatment and Group IV and group V received various doses of ethanolic leaf extract of *artemisia roxburghiana* plant daily once for 7 days(100mg/kg,400 mg/kg respectively).

Statistical analysis

After comparing the data using One-Way ANOVA, Tukey's multiple comparison test was run.GraphPad Prism version 5.0 was used to perform the statistical analysis. A p-value of less than 0.05 indicated that the statistics were significant.

Biochemical estimation:

Gathering of brain samples Following the cervical decapitation method of animal sacrifice, a brief anesthetic was administered, the whole mind was meticulously removed from the skull.A 10 ml formaldehyde solution was used to store the fresh whole brain(29). Hematoxylin and Eosin staining of a brain sample The brain section was divided into one section.After five minutes of heametoxylin staining, the slices were washed three times with tap water and blueing reagent.Following the rinse, the section were eosin-stained for ten minutes, and then they underwent two ethanol changes of 95% and 100% to dry them.After using xylene to clean the parts, coverslips were used to cover them(30).The eosin and heametoxylin stained hippocampi were examined under a light microscope and photo graphed taken of the slides by microscope(A)(31).

4.Results

Effect of *Artemisia roxburghiana* ethanolic extract on rats given scopolamine in a y-maze test

Effect of *Artemisia roxburghiana* on memory impairments brought on by scopolamine in the Y-maze test.Wistar rat model memory for spatial recognition was tested using the Y-maze test in relation regarding the consequences of the *artemisia roxburghiana* extract.whereby we examined the effect on the alterations in the behavior of rats.

Table 1 :Mean of % alterations in y maze test

Groups	Treatment	Doses mg/kg	Alterations %
Normal control	No treatment	No dose	64.09±2.650
Positive control	Scopolamine	1 mg/kg	47.71±3.992
Standard control	Piracetam	500mg/kg	62.16±2.804

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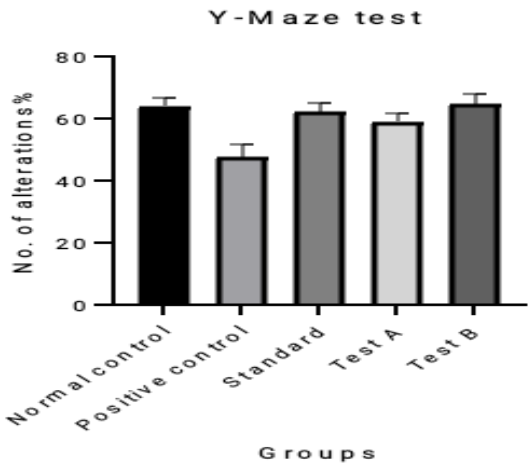
Test A	Extract of *artemisia roxburghiana*	100mg/kg	59.12±2.353
Test B	Extract of *artemisia roxburghiana*	400mg/kg	64.60±3.529

The mean is represented by values: The mean's standard error (n=6) and the significance level ($p<0.05$).

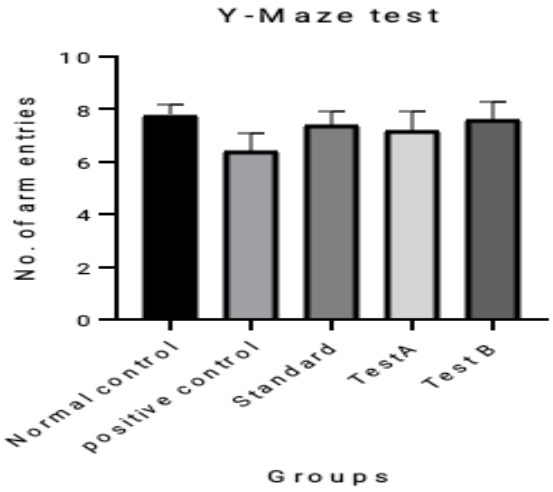
Effect on percentage alteration%

In contrast to the normal control group, the scopolamine control group displayed a significant ($p<0.01$) decrease in the altered behavior.*Artemisia roxburghiana* ethanolic extract pre-treatment (100 and 400 mg/kg body weight) did not significantly alter in response to contrast the normal control group; Nevertheless, in the instance of amnesia, the extract at a higher dose of 400 mg/kg body weight resulted in a noteworthy ($P<0.001$) alteration response (score: 64.60±3.529) in contrast to the other lower dose groups (score: 59.12±2.353, respectively), and it closelyresembledthe standard medication Piracetam (score: 62.16±2.804).

The spontaneous alternation results demonstrated that there was a notable difference ($P < 0.01$) between each treatment group.Rats with impaired spontaneous alternation were given scopolamine.In contrast to the scopolamine-alone treated group, a seven-day therapy with *artemisia roxburghiana* at all doses dramatically reversed the impact of scopolamine and raised the spontaneous alternation % ($P < 0.001$).Pre-administration of piracetam also undid the scopolamine-induced decrease in spontaneous alternation.Scopolamine at a percentage of 62.16% and the extract in a dosage of 400 mg/kg at a percentage of 64.60% were both counteracted by piracetam. All two extract doses, however, demonstrated improvement in the percentage alteration in a dose-dependent way.



Graph (1): No.of Alterations in y- maze test



Graph 2: No.of arm entries in Y- maze test

Effect of *Artemisia roxburghiana* ethanolic extract on rats given scopolamine in Locometer activity

With the digital actophotometer, locometer activity of ethanolic *Artemisia roxburghiana* is seen.*Artemisia roxburghiana* leaf extract in ethanolic form demonstrated nootropic effect by raising locometer activity.Scopolamine-treated positive control group's locometer activity was lower (119 ± 2.366)than that of the normal control group (143.8 ±7.910).The locometer activity (score: 144 ± 4.872) of the standard control group ranked greater than the amount of the positive control (scopolamine) group.The results of the *Artemisia roxburghiana* ethanolic leaf extract doses of 100 mg/kg (score: 134.8±7.692) and 400 mg/kg (score:

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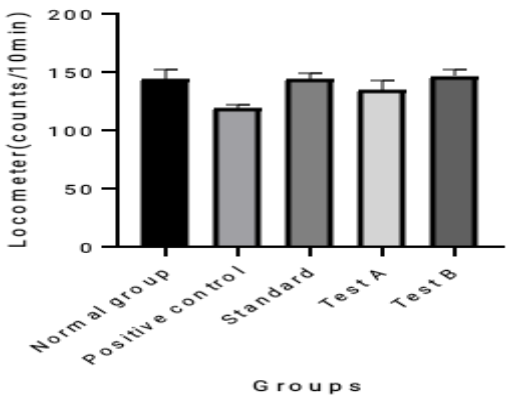
146.5±6.048) demonstrate an increase in locometer activity was compared opposed to the positive control group (scopolamine).Consequently, in comparison to the positive control group, the extract-treated groups had significantly ($p<0.001$) higher locometer activity.

Table2:Mean of Locometer activity (Counts/10 min)

Groups	Treatment	Doses mg/kg	Locometer activity counts (10min)
Normal control	No treatment	No dose	143.8 ±7.910
Positive control	Scopolamine	1mg/kg	119± 2.366
Standard control	Piracetam	500mg/kg	144 ± 4.872
Test A	Extract of artemisia roxburghiana	100mg/kg	134.8±7.692
Test B	Extract of *artemisia roxburghiana*	400mg/kg	146.5±6.048

The shown values are the mean (n = 6) plus the standard error of the mean. One way ANOVA was used for the study, and Tukey's multiple comparisons test was employed.Furthermore, it was determined that the "p value is indicated<0.05" was statistically significant.



Graph3:Locometer activity counts/ 10min

Histological examination

Figure4:Histology samples of rats brain

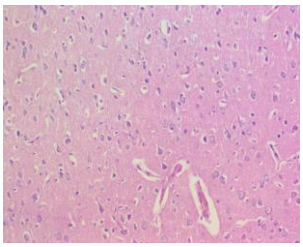


Figure4:(A)

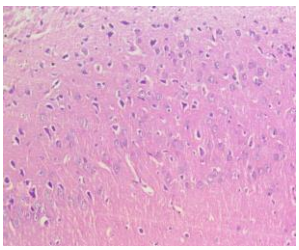


Figure 4:(B)

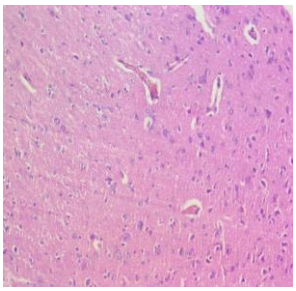


Figure4:(C).

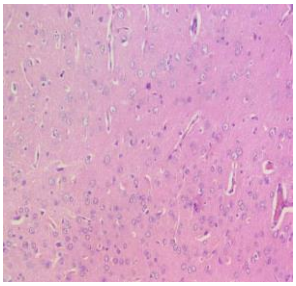


Figure4:(D)

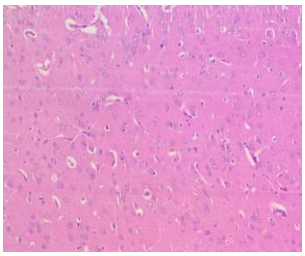


Figure4:(E)

Figure 4.) Eosin & hematoxylin staining of histopathology of brain tissues of rats brain

(A)Negative control(B)Positive control (C)Standardgroup(D)*Artemisia roxburghiana* 100 mg/ kg (E) *Artemisia roxburghiana* 400 mg/kg

Histopathological examination of rat's amnesia brought by scopolamine

A) Negative control (normal group):

Sections show brain parenchyma showing different brain structures like cerebral cortex,medulla,cerebral , ventricle and choroid plexus.All the parts of the brain are histologically remarkable.

Impression: Essentially normal histology

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B) Positive control:

Section shows Brain parenchyma showing different brain structure like cerebral cortex , medulla, cereballum, ventricle and choroid plexus.Cerebral cortex and other parts also showing moderate degenerative changes ,along with vascular congestion, moderate lymphoplasmatic inflitration and mild edema.Glial cells nuclei show anisokaryosis and reactive changes.

Impression: Mild gilosis and reactive changes

C) Standard treatment:

Section shows Brain parenchyma showing different brain structure like cerebral cortex , medulla, cereballum, ventricle and choroid plexus.Brain parenchyma show occasional degenerative changes along with vascular congestion and occasional lymphoplasmatic inflitration . Glial cells nuclei appearing almost uniform with no significant reactive changes.

Impression: Almost recovered brain parenchyma

D) Low dose of extract:

Section shows Brain parenchyma showing different brain structure like cerebral cortex , medulla, cereballum, ventricle and choroid plexus.Cerebral cortex and other parts also showing minimal degenerative changes ,along with mild vascular congestion , occasional lymphoplasmatic inflitration and minimal edema . Glial cells nuclei show occasional anisokaryosis and mild reactive changes.

Impression: Minimal gliosis

E) High dose of extract:

Section shows Brain parenchyma showing different brain structure like cerebral cortex , medulla, cereballum, ventricle and choroid plexus.Cerebral cortex and other parts also showing occasional degenerative changes ,along with minimal vascular congestion , occasional lymphoplasmatic inflitration and minimal edema.Glial cells nuclei show occasional anisokaryosis and minimal reactive changes.

Impression: Almost recovered gliosis .

The findings of the histopathological analysis are displayed in Figure 4. Figure 4(A)displays the normal control rat's histopathological section, which shows normal histology without any reactive changes.The scopolamine control group, on the other hand, exhibits neuronal degeneration, gliosis, vascular degeneration, and moderate edema in Figure 4(B).The results indicated that the standard treatment group (Figure 4(C), low-dose group (Figure 4(D), and high-dose group (Figure 4(E)) exhibited less gilosis, neuronal degeneration, and vascular degeneration than the negative control group.Group 2, which received scopolamine, showed the greatest abnormal alterations when compared to the standard groups, which were the ethanolic extract of *Artemisia roxburghiana* 400 mg group and the ethanolic extract of *Artemisia roxburghiana* 100 mg group both a little quantity and a high dose of ethanolic leaf extract.When the positive (scopolamine) control group was compared to the low and high doses of the ethanolic extract of *Artemisia roxburghiana*, both showed good regeneration scores.

5.Discussion

Every day, more and more people worldwide are being diagnosed with AD(32).Alzheimer's disease, which is explained by degenerative alterations in the brain, has memory loss as one of its primary symptoms (33).Scopolamine is an antimuscarnic medication that causes memory impairment (34).The well-known animal model of scopolamine-induced memory loss may be used by researchers to assess potential anti-amnestic medications (35).It effectively inhibits Ach muscarinic receptors and lowers Ach levels because it is an antagonist of non-selective muscarinic receptors. Piracetam increases Ach levels in the brain via binding to receptors.It does this via activating cholinergic receptors, which increases the synthesis of ACh.This implies that the brain receives more oxygen. Using herbal cognition boosters is a substitute for decreasing age-related cognitive decline and increasing attention spans Using an animal model of dementia, We looked into the consequences of an ethanolic extract of *Artemisia roxburghiana* on neurodegeneration and spatial memory.Since no prior research had assessed the nootropic potential of Artemisia roxburghiana leaves, the current investigation was carried out.The Y-maze test results show that there is a dose-dependent improvement in the percentage alteration change and a reduction in the quantity of arm entries in the event of scopolamine-induced amnesia. The findings clearly showed that when rats were given the Y-maze test, oral ethanolic extract of *Artemisia roxburghiana* given for seven days at doses of

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100 mg/kg and 400 kg/mg considerably improved the rats' Acquiring knowledge and recall Additionally, the locometer action in the scopolamine-induced wister rats was elevated by the *artemisia roxburghiana* extract.The locometer activity was higher in the ethanolic extract of *Artemisia roxburghiana* when dosages of 100 mg/kg and 400 mg/kg compared to the group that received scopolamine treatment.According to the locometer activity model, *Artemisia roxburghiana* extract showed increased nootropic activity.The *artemisia roxburghiana* extract shows good regeneration scores as compared to other groups after additional analysis using histological estimation, suggesting that it has a favorable nootropic impact on the scopolamine-induced wister rats.They have sufficient scientific worth to support the leaf extract from *Artemisia roxburghiana's* nootropic potential.

6.Conclusions

The following key conclusions were proposed by the thesis, "To explore nootropic activity of *artemisia roxburghiana* ethanolic leaf extract in Wister rats":

1.The ethanolic leaf extract of the *Artemisia roxburghiana* plant was prepared for this study and will be used to evaluate the nootropic activity using a soxhlet device.The study's findings about the nootropic effects of ethanolic extract are encouraging .

2.This study assessed a number of phytochemical assays and the bioactive properties of *Artemisia roxburghiana* samples collected from the northern region of the Chamoli district in Uttarakhand.The *Artemisia roxburghiana* extract exhibits positive results for flavonoids, glycosides, phenols, tannins, steroids, proteins, and carbohydrates in the initial phytochemical screening.

3.In the Y-maze test, the *Artemisia roxburghiana* ethanolic leaf extract demonstrated a higher percentage of increased% alterations changes and had more arm entries than the negative (scopolamine) control group.

4.The ethanolic extract of *Artemisia roxburghiana*, administered at doses of "100 mg/kg and 400 mg/kg," also increases locometer activity in comparison regarding the group of neurotic rats given a dose of 1 mg/kg of scopolamine.

5.Positive results are obtained from histological examination of brain tissues stained with eosin and hematoxylin.In comparison to the other groups and the scopolamine-induced group, the regenerative scores are higher in the therapy test groups.*Artemisia roxburghiana* groups A and B (100 mg/kg) and 400 mg/kg) in the H&E staining test exhibit good regeneration scores and less vascular degeneration and reactive alterations changes.

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