Neurobehavioral and neurochemical effects of *Passiflora caerulea* L. leaf extract on motor function in rats

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Abstract
The medicinal application of Passiflora species in Europe started in the 17th century; however, there has been limited research conducted on Passiflora caerulea L. This plant is quite adaptable, an herbaceous vine and is part of the Passifloraceae family. The neurobehavioral and neurochemical effects of extract from the leaves of Passiflora caerulea L. on rat motor function are investigated in this work. Assessing the extract's effects on central nervous system neurotransmitter levels, coordination and locomotor activity was the goal. After chronic oral administration of standardized extract doses (30, 100 and 300 mg/kg body weight) for a period of 47 days, behavioural evaluations such as the Novel Object Recognition (NOR) and Hole Board (HB) tests were carried out. A notable dose-dependent decrease in exploratory activity was noted, especially at higher extract doses, indicating sedative qualities, even if cognitive performance stayed constant.

HPLC-based neurochemical investigations showed significant monoaminergic system modification, with increased dopamine (DA) and its metabolites in the cerebellum and spinal cord, suggesting improved dopaminergic neurotransmission. These results support the extract's potential application in the treatment of motor dysfunctions and anxiety-related disorders by demonstrating its capacity to modify motor behaviour through dopaminergic and GABAergic pathways. To identify the active ingredients and completely clarify the molecular processes underlying these actions, more investigation is needed.

Keywords: Dopamine (DA), hole board (HB) tests, neurochemical effects, novel object recognition (NOR) test, monoaminergic, Passifloraceae.

Introduction

Passiflora caerulea L., popularly called as the blue passion flower, is a climbing vine native to South America and widely cultivated for its ornamental and medicinal properties. Traditionally, various species of *Passiflora* have been utilized in folk medicine for their sedative, anxiolytic and anticonvulsant effects. Recent pharmacological studies have begun to elucidate the neurochemical and neurobehavioral impacts of *Passiflora* species, particularly focusing on their potential to modulate central nervous system functions[6]. Neurological illnesses are a significant global healthcare burden, requiring creative therapeutic strategies. Plant-derived natural compounds have gained attention due to their diverse chemical structures and pharmacological actions[19].

Studies show their effectiveness in treating neurological disorders and relieving symptoms. These plants and their components have anti-inflammatory and antioxidant properties, protecting against neurodegeneration and other brain pathologies[7]. In terms of neurobehavioral effects, studies on related Passiflora species have provided insights into the potential impacts of *Passiflora caerulea*. For example, *Passiflora incarnata* has been demonstrated to enhance spatial memory and reduce anxiety in rats, likely through modulation of the GABAergic system and alterations in neurotransmitter levels such as serotonin and glutamic acid. Additionally, *Passiflora incarnata* has been reported to enhance neurogenesis and memory function in rodent models, suggesting a potential therapeutic role in cognitive disorders[9].

Bioactive substances such flavonoids, alkaloids and phenolic acids are part of the chemical makeup of Passiflora caerulea leaf extract and are thought to be involved in its pharmacological actions[3]. It has been discovered that its aerial parts, such as leaves and tendrils, possess antibacterial, gastroprotective, analgesic and anticonvulsant qualities. The plant's fruit extract has been shown to reduce seizure frequency and duration in animal models, providing a natural alternative to conventional seizure treatment. Numerous substances have aphrodisiac, antidepressant, antispasmatic, anticonvulsant, vermifuge, hypotensive, anticancer, diuretic, nervine sedative (anti-insomnia), anxiolytic, aphrodisiac, spasmolytic, analgesic, antibacterial, antifungal and antidepressant qualities.

Since the plant's leaves are more abundant than its blossoms, we looked into whether the leaves would also contain the same types of phytochemical components. We also measured some of the phytochemicals including alkaloids, flavonoids, tannins and phenolics[24]. Bioactive substances such as flavonoids (including chrysin, vitexin and luteolin), alkaloids, saponins, tannins and phenolic acids like 4-hydroxycinnamic acid are plentiful in *P. caerulea* leaves[3]. The potential therapeutic effects of these components are

acknowledged. The potential of *Passiflora caerulea* leaf extract as a useful natural medicinal agent is highlighted by its extensive pharmacological profile which includes antioxidant, anti-inflammatory, antibacterial, anticancer and neuroprotective qualities[6].

Although direct research on the consequences of *Passiflora caerulea* leaf extract on motor function in rats is limited, the existing evidence from related species and the known pharmacological properties of *Passiflora caerulea* suggest that it may influence motor behavior through its neurochemical actions. The current research explores the neurobehavioral and neurochemical effects of *Passiflora caerulea* leaf extract on motor function in rats, aiming to understand its potential therapeutic applications in neurological disorders and its mechanisms of neurotransmission alterations.

Material and Methods

Treatment of animals: We used male Wistar Albino Glaxo rats that were 4 weeks old and had a starting weight of the body of 51.33±2.45 g on average obtained from a certified breeding centre. For 6 weeks and throughout all behavioural procedures, all rats were given water *ad libitum* containing dry extract or drinking water (total extract administration time was 47 days). Chaw was made freely available to all the animals. These animals were maintained in pairs in typical plastic breeding cages in well-ventilated, air-conditioned space with a temperature of 22–24°C and a humidity of 55–10%. From 9:00 to 16:00, behavioural studies were carried out. Forty animals in all were employed in the experiment.

Four groups were randomly selected from among the animals: the standardized extract of *P. caerulea* leaf extract was administered to three groups of rats at doses of 30 mg (P30, n=10), 100 mg (P100, n=10) and 300 mg/kg b.w./day (P300, n=10) and control rats were given drinking water without restrictions (Con, n=10). The quantity of plant extract that corresponded to the preset each day doses was established by measuring the average daily fluid intake. Every day, a fresh solution was made[16].

Behavioral tests: Assessing the validity and reliability of animal models of neurodegenerative illnesses through behavioural testing enables researchers to look into the possible effectiveness of medication and other management strategies[29]. Behavioural tests, such as the Hole Board test (HB) and the Novel Object Recognition (NOR) test, were used to evaluate cognitive processes, anxiety and stress response and motor and exploratory activity. An observer who was blind to the conditions and design of the study evaluated the behaviour of the animals.

Novel Object Recognition (NOR) test: A wooden box was used to conduct the test for identifying the novel object measuring 100 cm by 100 cm by 35 cm. Animals were given a time of three minutes to familiarise themselves with the device before the test began. Two identical Lego bricks, designated A1 and A2, were placed in the box's opposing corners that are 10 cm away from the walls, on day 1 of the familiarisation phase. The objects were moved around during the second-choice phase and it was replaced by object B, a bottle of a different colour and form. Records were made of the amount of time spent investigating the objects overall, the number of contacts and the timing of the initial contact with the objects during the investigation. The difference in the time (t) spent discovering the new and familiar objects was used to compute the Discrimination Index (DI) for the choice phase [$DI = (T_N - T_F)/(T_N + T_F)$][14].

Additionally, the Global Habituation Index [GHI=(tA1+tA2)/(tB+tA1)], was also evaluated. It compares how long it takes to study two topics during the familiarization phase versus how long it takes during the decision phase. The Recognition Index (RI) considers the proportion of time spent looking at the novel item to time spent investigating the things in general [RI = tB/(tB+tA1)]. The aforementioned formulae were used to calculate the parameters DI, GHI and RI.

Hole Board (HB) test: A comparable box measuring 100 cm x 100 cm x 30 cm was used for the Hole Board test. There were sixteen squares (segments) on the apparatus floor. Every hole in the middle of the segments measured 3.8 cm in diameter and 5.0 cm in depth. The animals were each kept within the box for three minutes over the course of three days. Time spent in the apparatus's intermediate segments and while traveling, as well as metrics such as the head's penetration into the holes, ascent and transitions between the sectors, adjacent squares and outer sector of the test apparatus, were all recorded[28].

Data collection: A video camera captured every experiment. Blinding was used during the animal treatment phases, behavioural test behaviour analysis and result analysis. Every procedure was performed in accordance to the directives given by the Medical University of Warsaw's Ethical Committee for Animal Experiments and conducted in compliance with the guidelines set forth by the Vignan University.

Biochemistry – Sample preparation: After the animals completed the behavioural tests, the medulla oblongata, cerebellum and spinal cord were promptly sectioned on dry ice and beheaded with a guillotine. After the tissues were weighed, they were kept at -80°C until high-performance liquid chromatography (HPLC) was used to identify the neurotransmitters. Dopamine (DA) and its metabolites, homovanillic acid (HVA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), 3-methoxytyramine (3-MT), 3,4-dihydroxyphenyl acetic acid (DOPAC), are no longer present in the CNS structures. The ultimate neurotransmitter concentration is stated in ng/g of brain tissue[23].

Statistical analysis: To ascertain the importance of the

group differences, the Analysis of Variance (ANOVA) test was used. Post hoc tests were performed in the event that the null hypothesis was disproved. Depending on the Sedecor-Fisher F test results, either the Duncan (D) or Newman-Keuls (NK) tests were used. An Excel spreadsheet was used to generate the results, which were then displayed as mean ± SEM (mean standard error). Statistical significance was attained where the significance level (p) was below 0.05. TIBCO Statistica® 13.3 was used for all statistical studies.

Results

In order to study about the neurobehavorial and neurochemical effects of *Passiflora caerulea* L. leaf extract on motor function in rats, different tests were performed which include assessing behavioral tests such as Novel object Recognition tests and Hole Board tests. After successfully conducting the experiments, the following results were obtained. Animal groups did not differ in body weight over the course of the research ($F(3,36)=1.01$), or at the beginning ($F(3,36)=0.91$) and final ($F(3,36)=2.21$) body weights ($p>0.05$).

Novel Object Recognition (NOR) test: No discernible alterations in cognitive capacities were found. Rats' exploratory behaviour was not significantly altered by *Passiflora* extract during the familiarisation or testing phases with respect to control animals as shown in table 1. The global NOR parameters, including Global Habituation Index (GHI), the Recognition Index (RI), Discrimination Index (DI), did not differ significantly ($p>0.05$) as displayed in table 2.

Table 1 shows the results of a Novel Object Recognition test (mean ± SEM) in rats administered extract from *Passiflora caerulea* L. at doses of 30, 100 and 300 mg/kg b.w./day over a long duration (P30, n = 10; P100, n = 10; P300, n = 10) and control animals (Con, n = 10). To analyse group differences and determine their significance, ANOVA was utilized. Post-hoc tests were employed if the null hypothesis was disproved. The outcomes of the Duncan (D), Newman-Keuls (NK), or Snedecor-Fisher F tests were used.

Hole Board (HB) test: Rats given a larger extract dosage showed reduced mobility. In contrast to the P100 group and control group, the Analysis of Variance showed that rats given 300 mg dosages of extract experienced fewer crossings between segments over the course of the three-day research as shown in figure 1A. Comparing the other groups, the P300 group spent statistically less time in the middle parts of the apparatus, according to the HB test as shown in figure 1B. During the three days of the experiment, neither the number of climbs nor the number of head that dip into the holes, varied statistically significantly across the groups ($p>0.05$). All of the rat groups under study had comparable motor activity, or the amount of time spent moving.

Concentration of metabolites and monoamines in specific CNS structures (ng/g tissue): Monoamine and metabolite levels alter significantly, affecting the dopaminergic and noradrenergic systems. Following prolonged treatment of *P. caerulea* extract, the neurotransmitter concentration in the rats' spinal cord, medulla and cerebellum changed. The amount of 5-HT metabolite 5-HIAA present in the brain was found to have changed significantly. In comparison to the control group, the 5-HIAA concentration was less prevalent in the group that received the *P. caerulea* L. extract. However, compared to control animals, the groups receiving the extract had higher levels of 5-HIAA in their spinal cords ($F(3,36)=2.78$; $p<0.05$). The P30 and P300 groups had higher spinal cord serotonin (5-HIAA/5-HT) turnover than the control group ($F(3,36)=5.96$; $p<0.005$). The concentration of NA in the cerebellum varied significantly, according to Analysis of Variance ($F(3,36)=5.99$; $p<0.005$).

Furthermore, there were notable variations in the spinal cord's levels of the NA metabolite MHPG ($F(3,36)=4.88$, $p<0.01$). In the CNS structures that were analysed, the groups which received extract, showed a greater concentration of DA than the control group as in figure 2 A.

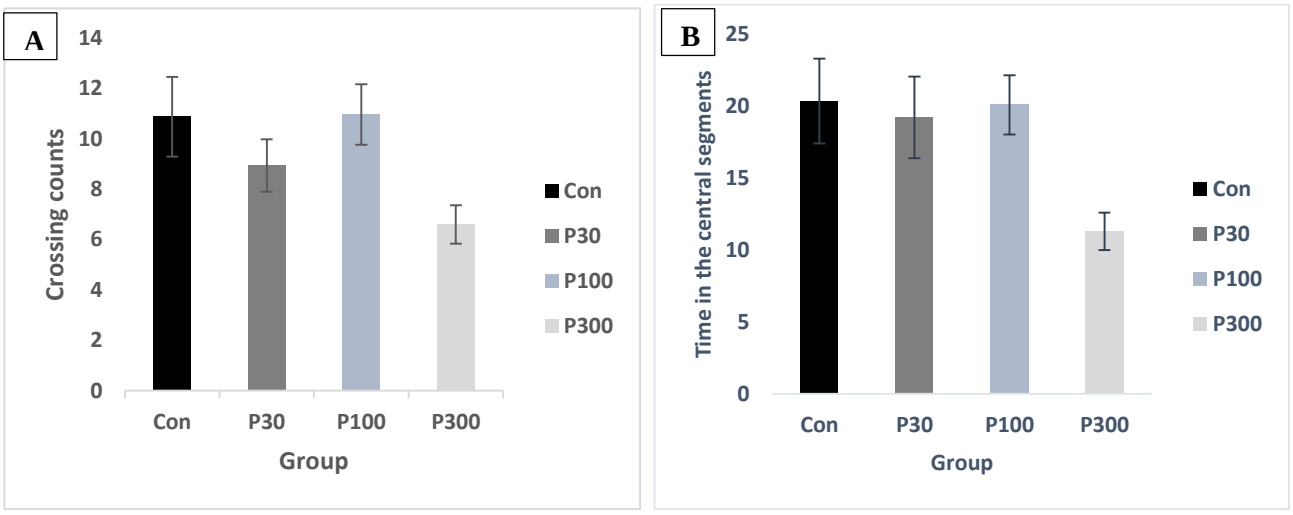

Figure 1: Hole Board Test

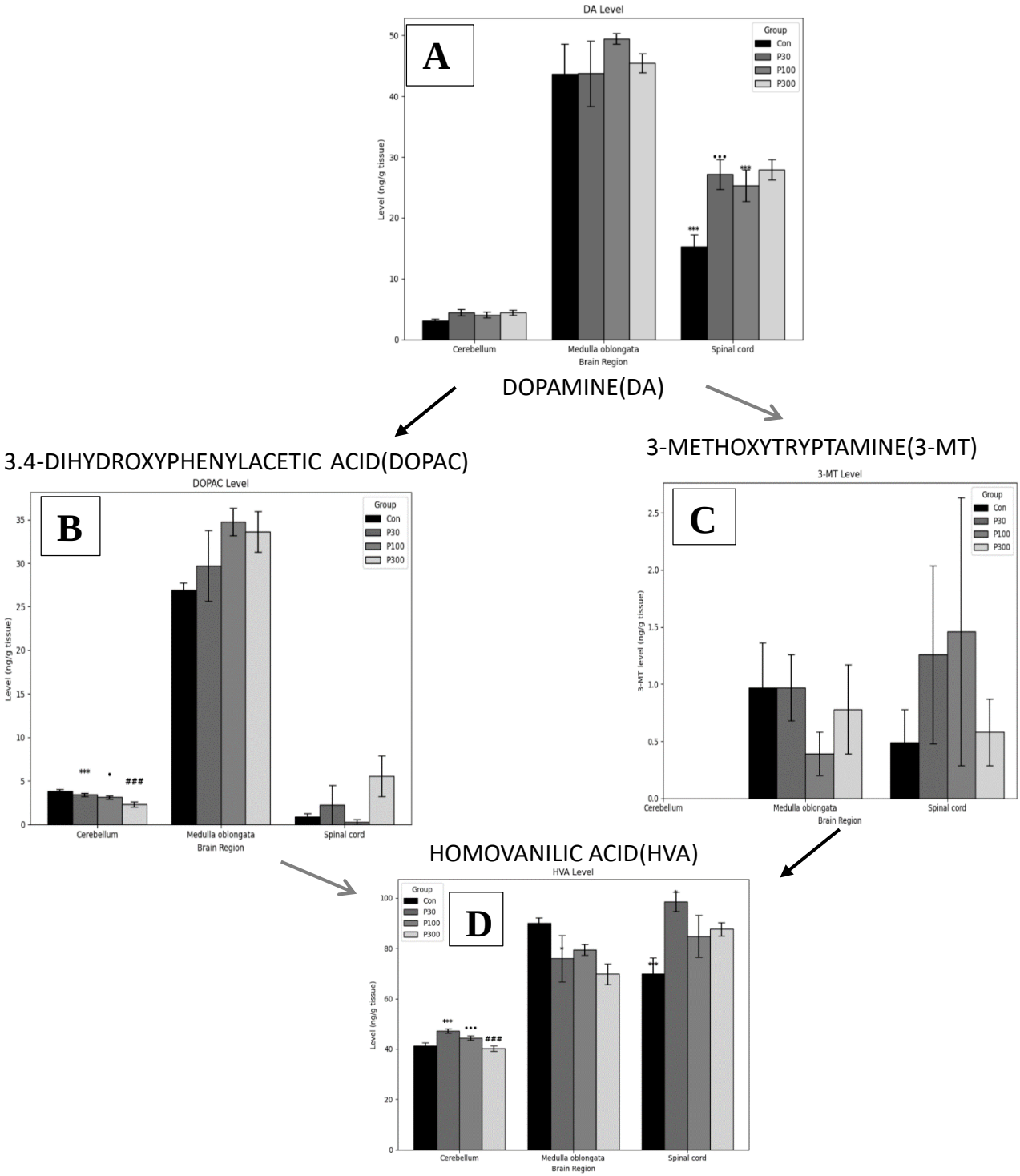

Figure 2: Concentration of metabolites and monoamines in specific CNS structures.

Table 1
Novel Object Recognition (NOR) test

	Latency to first contact with object (s)		No. of contacts with object		Total time spent examining objects (s)	
Group	**Latency A1 (s)**	**New object B**	**A1 Contacts**	**B Contacts**	**A1 Time (s)**	**B Time (s)**
Con	16.05 ± 3.04	30.5±13.25	4.48 ± 0.49	3.21 ± 0.39	19.36 ± 4.05	12.75 ± 5.8
P30	25.69 ± 13.01	18.1±6.05	3.79 ± 0.50	2.92 ± 0.48	12.55 ± 2.21	7.98 ± 1.46
P100	17.61 ± 3.06	18.5±8.70	3.89 ± 0.32	3.70 ± 0.61	13.04 ± 2.33	8.27 ± 1.72
P300	18.49 ± 6.31	10.4±4.56	5.35 ± 0.65	3.60 ± 0.51	13.52 ± 1.94	6.42 ± 1.06

Table 2
Global NOR Parameters

Global NOR Parameters				
Group	**Total Exam Time (s)**	**GHI**	**RI**	**DI**
Con	50.6 ± 6.82	0.76 ± 0.14	0.36 ± 0.07	-0.25 ± 0.14
P30	36.28 ± 3.89	0.8 ± 0.06	0.39 ± 0.06	-0.19 ± 0.12
P100	40.08 ± 3.48	1.05 ± 0.21	0.37 ± 0.07	-0.24 ± 0.14
P300	36.49 ± 3.76	0.84 ± 0.08	0.32 ± 0.05	-0.33 ± 0.10

Table 3
Monoamine Turnover

Monoamine Turnover				
Region	**Group**	**5-HIAA/5-HT**	**DOPAC/DA**	**HVA/DA**
Cerebellum	Con	0.74 ± 0.05	1.30 ± 0.17	13.71 ± 1.11
	P300	0.60 ± 0.04	0.55 ± 0.08	9.20 ± 0.68
Spinal Cord	Con	0.36 ± 0.02	0.10 ± 0.05	5.06 ± 0.72
	P300	0.46 ± 0.03	0.21 ± 0.09	3.16 ± 0.23

The spinal cord's DA concentration varied significantly between animal groups ($F(3,36)=6.99$, $p<0.005$). The levels of the DA metabolite DOPAC in the cerebellum ($F(3,36)=6.40$; $p<0.005$) and HVA ($F(3,36)=9.91$; $p<0.005$) varied significantly, according to Analysis of Variance as in figure 2B and 2C. Furthermore, there were notable variations in the levels of HVA in the spinal cord ($F(3,36)=4.30$, $p<0.01$) and the medulla oblongata ($F(3,36)=2.68$; $p<0.05$) as in fig. 2D.

All groups that received extract, had lower cerebellar DA turnover ($F(3,36)=6.15$; $p<0.005$). Notable distinctions in the cerebellum's DA turnover (DOPAC/DA) were revealed by ANOVA. Significant variations were also noted in the HVA/DA turnover in the spinal cord ($F(3,36)=3.98$; $p<0.05$) and cerebellum ($F(3,36)=3.27$; $p<0.05$) for HVA, another DA metabolite.

Monoamine Turnover: In the cerebellum, the 5-HIAA/5-HT ratio dropped from 0.74 ± 0.05 to 0.60 ± 0.04 in the P300 group, indicating a 19% decrease in serotonin turnover. The DOPAC/DA ratio decreased by approximately 58%, from 1.30 ± 0.17 to 0.55 ± 0.08, indicating a considerable suppression of intracellular dopamine metabolism. The HVA/DA ratio decreased by almost 33% (from 13.71 ± 1.11 to 9.20 ± 0.68), indicating decreased extracellular dopamine turnover. These data suggest that both serotonergic and dopaminergic metabolism in the cerebellum are downregulated under P300 circumstances as shown in table 3.

In contrast, the spinal cord demonstrated a more complicated pattern. Serotonin turnover, measured by 5-HIAA/5-HT, rose by 28% (from 0.36 ± 0.02 to 0.46 ± 0.03), indicating higher serotonergic activity in this location. The DOPAC/DA ratio increased from 0.10 ± 0.05 to 0.21 ± 0.09, indicating higher early dopamine metabolism. However, the HVA/DA ratio reduced dramatically (-38%, from 5.06 ± 0.72 to 3.16 ± 0.23), indicating that early-stage dopamine breakdown increased while progression to its extracellular metabolite decreased. This separation of early and late metabolic processes suggests a specific shift in dopamine catabolic pathways.

Discussion

Passiflora caerulea is a versatile herbaceous plant which belongs to the *Passifloraceae* family. Initially it was only used as a sedative agent but currently its pharmacological application and functionality have been the main subjects of scientific investigations[6]. The study's objective was to assess how *Passiflora caerulea* leaf extract influences the neurobehavioral and neurochemical effects on motor function in rats. The study explores the mechanisms behind the decrease in motor hyperactivity following *P.caerulea* administration by analysing neurotransmission alterations in motor function-related structures like the cerebellum, spinal cord and medulla oblongata. Determining the impact of administering *P. caerulea* L. extract on rats' anxiety levels in addition to their exploratory and motor activities, was one of the goals of the study.

Neither the initial nor end body weights of the animal groups varied during the duration of the investigation. Rats given a lower dose of extract spent noticeably less time spent in the centre of the Hole Board apparatus during the trial, while rats given a greater dose had fewer passes between segments and decreased mobility compared to the control group. This could suggest that high dosages of the extract have a sedative or relaxing effect. The study's findings are consistent with information gathered by other researchers in a variety of rodent behavioural tests.

Animals administered pentobarbital exhibited a calming effect in the staircase test (fewer climbing and ascents), a reduction in motor activity in the free exploration test and an extension of sleep, based on an examination of the 400

mg/kg water extract from the Passiflora plant's aerial parts' central effect[25].

Some *Passiflora* species have been reported to exhibit anti-inflammatory, anti-obesity potential and antinociceptive qualities, as well as anxiogenic, hypoglycemic activity, anticonvulsant effects, sedative qualities, photoprotective activity, antibacterial efficacy, cytotoxic effects[18]. In an HRBC membrane-stabilization assay, Sindhura and Bobby's[24] study revealed that the *P. caerulea* leaf extract (800 mg/mL) significantly reduced inflammatory activity, measuring 61.05 ± 1.5%. The efficacy of the leaf extract was highlighted by the fact that aspirin, the positive control, showed an inhibition rate of 90.41 ± 1.3% at the same concentration. The findings of Sindhura and Bobby's[24] investigation show that the *P. caerulea* extract has a strong anti-breast cancer (MCF-7) cell effect.

The plant's crude methanolic extract showed significant action against cells of breast cancer (MCF-7), according to the results of the MTT experiment. After observing dose-dependent cellular viability, the IC50 was determined to be 50.22 µg/mL. These findings call for more research on the pharmacological and therapeutic potential of P. *caerulea* extracts. Three Passiflora species: *P. alata*, *P. caerulea*, *P. incarnata*, were evaluated by Ozarowski et al[17] for their ability to inhibit human acute lymphoblastic leukaemia CCRF-CEM cells.

The impact of Passiflora species on the nervous system is one of their most researched characteristics. The acetylcholinesterase activity as well as the anticonvulsant, anxiolytic and neuro-modulatory properties of extracts from *Passiflora caerulea* have been the subject of several investigations. The acetylcholinesterase inhibitory potential of *P. caerulea* has been conducted by Aseervatham et al[1] which is significant when considering neurological illnesses like Alzheimer's disease. The brain enzyme acetylcholinesterase breaks down the neurotransmitter acetylcholine, which is essential for cognitive function. When this enzyme is inhibited, more acetylcholine is produced, which affects memory and cognitive issues.

Numerous components of *Passiflora caerulea* including flavonoids, phenolic compounds and alkaloids, have been shown in phytochemical investigations to have an AChE inhibitory action. Aseervatham et al's[1] study found that the Pilocarpine-induced mice's hippocampus after receiving extract from Passiflora had lower levels of AChE (0.35 ± 0.86 µmol/min/mg protein) than the control group. According to the same authors, *P. caerulea* extract has anticonvulsant properties that also affect cognitive function and lessen oxidative damage[1]. Using *in vitro* tests, Medina et al[15] verified the anxiolytic properties of chrysin, which was extracted from *P. caerulea's* aerial parts.

Clinical research has demonstrated that *P. caerulea* extract has sedative and anxiolytic properties that help to alleviate anxiety and sleeplessness by lowering tension, restlessness and nervous irritability. Numerous researchers have compared Passiflora extract to traditional anxiolytic medications, indicating that it could be used as an adjuvant or alternative therapeutic option.

One of the examinations used to evaluate the cognitive abilities of animals is the Novel Object Recognition test (NOR), the second behavioral test carried out throughout the study. The examination looks at cognitive function, object recognition skills and novelty preferences. Animals are said to be more inclined to investigate new objects and novelty preference indicates that the animal's memory still has the image of the previous thing[4]. Manual skills and habits are the focus of procedural (non-declarative) memory, which function outside of consciousness. It is located in the amygdala, striatum and cerebellum and is learnt through conditioning and motor functions[10]. The primary metrics describing the animals' behavior in this test did not exhibit any discernible variations.

Rats given *P. caerulea* L. extract and the control group both spent comparable amounts of time inspecting both new and old items. We may conclude that the extract does not interfere with associative and cognitive functioning, in contrast to several regularly used anti-anxiety medications. The group receiving the greatest dose of the extract had more total interactions with both objects throughout the choosing phase, which was the only difference between the groups.

The presence of a novel object might alter an animal's behavior by stimulating interest or causing a stress response. It is commonly recognized that stress causes NA to be released by the sympathetic nervous system which is crucial for controlling the stress response and governing memory and learning. Stress-induced increases in NA release impair memory by changing how the prefrontal cortex functions[26]. The findings suggest that the noradrenergic system mediates stress-induced learning and memory impairment. It is also recognized that medications that lessen the noradrenergic system's excessive activity may be helpful in treating disorders like anxiety and post-traumatic stress disorder (PTSD) that are linked to stress.

The cerebellum, a region of the central nervous system involved in balance and motor coordination, showed a decrease in NA concentration in animals given P. *caerulea* L. extract; however, these changes in the neurotransmitter's level did not result in changes in memory or object recognition. It is thought that NA, a significant neuromodulator in the cerebellum, controls synaptic transmission and the integration of sensory information in the cerebellar cortex's granular layer[12].

Sun et al[27] discovered that by stimulating molecular layer interneurons in mice, NA prevented the cerebellar Purkinje cells from becoming less active. In addition to being essential for movement and coordination, the cerebellum

collaborates closely with the cerebral cortex and has the ability to influence non-motor processes. According to some researchers, reducing NA levels often causes an increase in NA release and mitigates the negative effects of stress that come with behavioral testing[22]. This is supported by both the current study, which found a drop in the amount of NA in the cerebellum and earlier research that demonstrated the positive effects of *P. caerulea* leaf extract on the stress response and the corresponding decrease in NA concentration in the prefrontal cortex.

The healthy musculoskeletal structure and function, as well as the nervous system's ability to regulate activity, are pre-requisites for motor development. Both vertebrates' and invertebrates' locomotor system activation and modification are significantly influenced by the dopaminergic system which regulates the spinal cord motor circuit[8]**.** According to the research, DA shapes the locomotor system during development and influences the transition of motor abilities and behaviour from immature to more mature movement patterns[11]. It affects behavior and motor abilities, leading to motor issues such as restless legs syndrome and Parkinson's disease.

In all examined CNS areas, a significant impact of the extract on DA metabolism and dopaminergic neurotransmission was discovered in the study. In animals given *P. caerulea* L. extract, there was an increase in the amount of DA in the spinal cord and its metabolite HVA. Additionally, the extract reduced DA turnover in the spine and brain and significantly altered the amount of DA metabolites in the medulla oblongata and cerebellum. Many studies have examined *Passiflora caerulea's* anxiolytic (anxiety-reducing) properties. One potential mechanism for *P. caerulea's* anxiolytic effects is through modulation of the GABAergic system, where gamma-aminobutyric acid (GABA) is a neurotransmitter that is essential for preventing excessive neuronal activity, which reduces anxiety and promotes relaxation.

It has been shown that various compounds in *P. caerulea* may improve GABAergic transmission by using benzodiazepine-like modes of action with fewer adverse effects[5]. For instance, it has been discovered that the flavonoid chrysin binds to GABA receptors, boosting anxiolytic effect[21]. The alkaloids harmane and harmine exhibit calming effects because they can alter neurotransmitter networks[13]. The aphrodisiac properties of *P. caerulea* have been demonstrated by Dhawan et al[2]. The suppression of aromatase, which converts androgens to oestrogens, may be an underlying component.

According to the study, animals' cognitive function improved when the extract was administered, as evidenced by an increase in object recognition. In the current investigation, animals given P. *caerulea* L. extract over an extended period of time showed increased levels of DA in their spinal cords. All five dopamine receptor subtypes, which are irregularly distributed throughout the spinal cord, have been demonstrated to be impacted by dopamine (DA) exuded from rats' lumbar spinal cords neuronal terminals[30]. DA promotes the development of new nerve cells, which includes adult and embryonic neurogenesis in spinal cord motor neurones[20].

P. caerulea L. extract treatment had no effect on the 5-HT level in CNS tissues in the current study; nevertheless, minor alterations in the amount of this neurotransmitter's metabolites and 5-HT conversion in the cerebellum and spinal cord to 5-HIAA were observed. 5-HT turnover and 5-HIAA levels in the spine increased in rats given the extract at doses of 30 and 300 mg. On the other hand, 5-HIAA cerebellum levels significantly decreased following dosages of 100 and 300 mg of the extract. The study discovered that under P300 settings, the cerebellum and spinal cord had a considerable drop in serotonin turnover, DOPAC/DA ratio and HVA/DA ratio, indicating a downregulation of both serotonergic and dopaminergic metabolism.

However, the spinal cord demonstrated an increase in serotonin turnover, DOPAC/DA ratio and HVA/DA ratio, indicating a specific modification in dopamine catabolic pathways. Such region-specific changes in turnover ratios indicate that monoamine metabolism can be controlled separately in different CNS areas depending on the treatment or state. P300 suppresses both serotonin and dopamine catabolism in the cerebellum, but has a more nuanced effect in the spinal cord, with differing effects on early and late dopamine processing steps. This combination of reduced cerebellar turnover and selectively altered spinal cord metabolism could have functional consequences, perhaps impacting region-specific neuronal signalling, plasticity, or behaviour.

Conclusion

In conclusion based on the above evidences, *Passiflora caerulea* appears to have notable neuro-modulatory and neuroprotective properties, including mild GABAergic inhibition, dopaminergic enhancement, anticonvulsant, analgesic effects. The current work demonstrates that extract from *P. caerulea* L. modifies neurotransmission in key CNS locomotor system components. Disorders involving a deficiency in dopaminergic neurotransmission may benefit from a substantial elevation in the amount of dopamine (DA) in the spinal cord. The extract from Passiflora may have sedative properties and a potential substitute for benzodiazepines in the treatment of anxiety and hyperactivity because of its inhibitory action on the GABAergic system. These results may result in the creation of certain supportive therapies to help reduce anxiety and symptoms associated with a progressive loss or impairment of motor behaviour.

P. caerulea has the potential to be a mild substitute for traditional sedatives or anxiolytics by gently boosting dopamine pathways and promoting GABA receptor

activation. Its anti-inflammatory and antioxidant properties may also help protect motor and cognitive abilities. To determine the ideal dosage, validate safety and thoroughly establish its therapeutic efficacy in neurological diseases, anxiety and motor-related impairments, thorough mechanistic research and clinical trials are necessary.

References

1. Aseervatham G.S.B., Abbirami E., Sivasudha T. and Ruckmani K., Passiflora caerulea L. fruit extract and its metabolites ameliorate epileptic seizure, cognitive deficit and oxidative stress in pilocarpine-induced epileptic mice, *Metabolic Brain Disease*, **35**, 159–173 **(2019)**

2. Dhawan K., Dhawan S. and Sharma A., Passiflora: a review update, *Journal of Ethnopharmacology*, **94(1)**, 1–23 **(2004)**

3. El-Askary H.I., Haggag M.Y., Abou-Hussein D.R., Hussein S.M. and Sleem A.A., Bioactivity-guided study of Passiflora caerulea L. leaf extracts, *Iranian Journal of Pharmaceutical Research (IJPR)*, **16(Suppl)**, 46 **(2017)**

4. Ennaceur A., One-trial object recognition in rats and mice: methodological and theoretical issues, *Behavioural Brain Research*, **215(2)**, 244–254 **(2010)**

5. Feliú-Hemmelmann K., Monsalve F. and Rivera C., Melissa officinalis and Passiflora caerulea infusion as physiological stress decreaser, *International Journal of Clinical and Experimental Medicine*, **6(6)**, 444 **(2013)**

6. Garmidolova A., Halkoglu-Hristova P. and Georgiev V., Exploring the multifunctionality of Passiflora caerulea L.: From traditional remedies to modern applications, *Applied Sciences*, **15(6)**, 3251 **(2025)**

7. Haobam R., Nongthombam P.D. and Tongbram Y.C., Importance of traditional medicines in the treatment of neurological diseases, In Traditional Resources and Tools for Modern Drug Discovery: Ethnomedicine and Pharmacology, Springer, Nature, Singapore, 527–550 **(2024)**

8. Hosseini P., Mirsadeghi S., Rahmani S., Izadi A., Rezaei M., Ghodsi Z., Rahimi-Movaghar V. and Kiani S., Dopamine receptors gene expression pattern and locomotor improvement differ between female and male zebrafish during spinal cord auto repair, *Zebrafish*, **19(4)**, 137–147 **(2022)**

9. Jawna-Zboińska K., Blecharz-Klin K., Joniec-Maciejak I., Wawer A., Pyrzanowska J., Piechal A., Mirowska-Guzel D. and Widy-Tyszkiewicz E., Passiflora incarnata L. improves spatial memory, reduces stress and affects neurotransmission in rats, *Phytotherapy Research*, **30(5)**, 781–789 **(2016)**

10. Kandel E.R., Dudai Y. and Mayford M.R., The molecular and systems biology of memory, *Cell*, **157(1)**, 163–186 **(2014)**

11. Lambert A.M., Bonkowsky J.L. and Masino M.A., The conserved dopaminergic diencephalospinal tract mediates vertebrate locomotor development in zebrafish larvae, *Journal of Neuroscience*, **32(39)**, 13488–13500 **(2012)**

12. Li B.X., Jin H., Zhang G.J., Cui L.N., Chu C.P. and Qiu D.L., Effect of noradrenaline on the facial stimulation-evoked mossy fiber-granule cell synaptic transmission in mouse cerebellar cortex, *Frontiers in Neuroscience*, **15**, 785995 **(2021)**

13. Li S.P. et al, Analogous β-carboline alkaloids harmaline and harmine ameliorate scopolamine-induced cognition dysfunction by attenuating acetylcholinesterase activity, oxidative stress and inflammation in mice, *Frontiers in Pharmacology*, **9**, 346 **(2018)**

14. Lueptow L.M., Novel object recognition test for the investigation of learning and memory in mice, *Journal of Visualized Experiments (JoVE)*, **126**, 55718 **(2017)**

15. Medina J.H., Paladini A.C., Wolfman C., Levi de Stein M., Calvo D., Diaz L.E. and Peña C., Chrysin (5,7-di-OH-flavone), a naturally-occurring ligand for benzodiazepine receptors, with anticonvulsant properties, *Biochemical Pharmacology*, **40(10)**, 2227–2231 **(1990)**

16. Mirowska-Guzel D. and Widy-Tyszkiewicz E., Effect of Passiflora incarnata L. extract on exploratory behaviour and neurotransmitter levels in structures involved in motor functions in rats, *Journal of Pre-Clinical and Clinical Research*, **18(1)**, 1–10 **(2024)**

17. Ozarowski M. et al, Comparison of bioactive compounds content in leaf extracts of Passiflora incarnata, P. caerulea and P. alata and in vitro cytotoxic potential on leukemia cell lines, *Revista Brasileira de Farmacognosia*, **28(2)**, 179–191 **(2018)**

18. Pereira Leal A.E.B., De Lavor É.M., Barbosa J.M., Araújo M.T.M.F., Alves C.S.C., De Oliveira Júnior R.G., De Lima Á.A.N. and Almeida J.R.G.S., Pharmacological activities of the genus Passiflora (Passifloraceae): a patent review, *Current Topics in Medicinal Chemistry*, **22(28)**, 2315–2328 **(2022)**

19. Prakash S. and Verma S., Harnessing the therapeutic potential of plant-derived natural products in neurological disorders, *Journal of Drug Discovery and Health Sciences*, **1(1)**, 28–33 **(2024)**

20. Reimer M.M. et al, Dopamine from the brain promotes spinal motor neuron generation during development and adult regeneration, *Developmental Cell*, **25(5)**, 478–491 **(2013)**

21. Rodríguez-Landa J.F. et al, Bernal-Morales B. and Herrera-Huerta E.V., Chrysin reduces anxiety-like behavior through actions on GABAA receptors during metestrus-diestrus in the rat, *Behavioural Brain Research*, **397**, 112952 **(2021)**

22. Scullion G.A., Kendall D.A., Sunter D., Marsden C.A. and Pardon M-C, Central noradrenergic depletion by DSP-4 prevents stress-induced memory impairments in the object recognition task, *Neuroscience*, **164(2)**, 415–423 **(2009)**

23. Shao X.M. and Feldman J.L., Efficient measurement of endogenous neurotransmitters in small localized regions of central nervous systems in vitro with HPLC, *Journal of Neuroscience Methods*, **160(2)**, 256–263 **(2007)**

24. Sindhura L. and Bobby M.N., Phytochemical profiles, antioxidant, antimicrobial and cytotoxic cell lines activity of Passiflora caerulea L., *Biomedical & Pharmacology Journal*, **15(4)**, 2365–2379 **(2022)**

25. Soulimani R., Younos C., Jarmouni S., Bousta D., Misslin R. and Mortier F., Behavioural effects of Passiflora incarnata L. and

its indole alkaloid and flavonoid derivatives and maltol in the mouse, *Journal of Ethnopharmacology*, **57(1)**, 11–20 **(1997)**

26. Srikumar B.N., Raju T.R. and Rao B.S.S., The involvement of cholinergic and noradrenergic systems in behavioral recovery following oxotremorine treatment to chronically stressed rats, *Neuroscience*, **143(3)**, 679–688 **(2006)**

27. Sun N., Li B.X., Hong Y.J., Bing Y.H., Qiu D.L. and Chu C.P., Noradrenaline depresses spontaneous complex spikes activity of cerebellar Purkinje cells via α2-adrenergic receptor *in vivo* in mice, *Neuroscience Letters*, **703**, 38–44 **(2019)**

28. Takeda H., Tsuji M. and Matsumiya T., Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice, *European Journal of Pharmacology*, **350(1)**, 21–29 **(1998)**

29. Tello J.A., Williams H.E., Eppler R.M., Steinhilb M.L. and Khanna M., Animal models of neurodegenerative disease: recent advances in fly highlight innovative approaches to drug discovery, *Frontiers in Molecular Neuroscience*, **15**, 883358 **(2022)**

30. Zhu Q., Mao L.N., Liu C.P., Sun Y.H., Jiang B., Zhang W. and Li J.X., Antinociceptive effects of vitexin in a mouse model of postoperative pain, *Scientific Reports*, **6(1)**, 19266 **(2016)**.

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