RESEARCH PAPER

Effect of Montelukast on Anxiety-Related Behavioural Functions in Albino Rats

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ABSTRACT

Background: Montelukast is used to treat exercise-induced bronchoconstriction, allergic rhinitis, and asthma. Montelukast's post-marketing safety signals have revealed neuropsychiatric side effects, including suicidal thoughts and actions, anxiety, sadness, anger, and sleep issues.

Aim: The main aim of the study was to evaluate anxiety like effects of Montelukast in albino rats.

Methods: The study was conducted in albino rats after obtaining approval from Institutional Animal Ethics Committee, KIMS Bhubaneswar & the study period was for 10 days. About 24 albino wistar rats weighing 150– 200g were split up into 4 groups, with each group containing 6 animals. The groups received saline as Control, Montelukast 10mg/kg and 20mg/kg as test drug, Caffeine 50mg/kg as standard drug intraperitoneally. Evaluation of anxiety effects was done using Open field test, Elevated Plus Maze Test and social interaction test.

Results: The groups receiving Montelukast showed anxiety like effects on the rodent models of anxiety.

Conclusion: Montelukast has shown anxiety like effect in experimental rats. Further studies are required on humans to authenticate this claim.

Keywords: *Montelukast, Albino Wistar rat, Anxiety*

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1. INTRODUCTION

Anxiety is a psychological condition characterized by excessive and unwanted fear or apprehension that affects an individual's thoughts and behaviour. Anxiety disorders affected 359 million individuals worldwide in 2021, accounting for the most prevalent mental disorder in modern society. Currently, 4.4% of people worldwide suffer from anxiety disorders *[1]*. Although anxiety is a common human response and not a disease by itself, it is closely linked with various psychiatric disorders. Experimental studies using animal models have demonstrated that several environmental and physiological factors can provoke anxiety like behaviour [2]. These factors include exposure to height, hunger, isolation, illness, thirst, threatening situations, violence, pain, darkness and various physical stimuli such as electric current, heat, cold, pinching and alternations in self perception. Neurobiologically, the reticular formation of the brain essentially plays a significant role in the regulation of anxiety states. In certain people, anxiety may be associated with an underlying health issue like heart disease, diabetes, thyroid problems, such as hyperthyroidism. In few individuals, anxiety can be a side effect of certain medications used in respiratory disorders, such as chronic obstructive pulmonary disease (COPD) and asthma [3]

Montelukast is a cysteinyl leukotriene receptor 1 (CysLT1) antagonist and is widely used for the prophylactic and chronic treatment of asthma, symptomatic relief of allergic rhinitis and acute prevention of exercise-induced bronchoconstriction. Obstructive sleep apnea and chronic obstructive pulmonary disease are two off-label applications of montelukast [4]. Since its introduction into the market in 1998, an increase in adverse drug reactions (ADRs), especially neuropsychiatric side effects, has been attributed to montelukast. Nightmares, general anxiety, aggression, sleep difficulties, sleeplessness, irritability, hallucinations, hyperactivity, and personality abnormalities are the most typical manifestations of these ADRs related to montelukast [5]. Aggression, anxiety, depression, various sleep-related problems, suicidal ideation, self-harm, and completed suicide have been reported as severe neuropsychiatric symptoms associated with the use of montelukast. These adverse effects have been documented in post-marketing surveillance as well as in several case reports, particularly in children [6].The US Food and Drug Administration issued a warning in 2008

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regarding montelukast's neuropsychiatric adverse effects. The warnings and precautions section of Singulair®(montelukast sodium) was updated in 2009 to encompass neuropsychiatric occurrences. In 2020, the US Food and Drug Administration (FDA) issued a black box warning concerning potentially significant mental health outcomes attributed to montelukast use and suggested reducing its use in the treatment of allergic rhinitis [7].

There is no scientific information to demonstrate the effect of montelukast on the behavioural changes occurring in experimental animals. This study is aimed to evaluate the anxiogenic effects of montelukast in albino rats, and to assess this effect with standard medication like caffeine which is used to produce anxiety.

2. MATERIALS & METHODS

After acquiring permission of the Institutional Animal Ethics Committee (IAEC No. 1730/PO/Re/13/CPCSEA), the study was accomplished inside the Animal House at the KIMS Department of Pharmacology. The study period was 60 days.

Experimental animals

About 24 healthy adult albino Wistar rats of either sex, weighing between 150-200g, have been collected in the study. The animals were kept in polypropylene cages covered with metal grid and fed with standard laboratory diet and water *ad libitum*. They were kept within controlled room temperature (24± 2° C) and humidity (55± 5%) following a 12hr light/dark cycle. All necessary measures were employed to mitigate the animals' pain and discomfort during the experimental procedures.

Drugs and Chemicals

The drugs montelukast (Cipla Ltd.) and caffeine (Nutrabay) in tablet form were obtained from KIMS pharmacy. The chemicals required for the experiment were purchased from a nearby shop and stored under suitable conditions.

Acute Toxicity Study

In accordance with the guidelines set by the Organisation for Economic Cooperation and Development (OECD), specifically guideline No. 423, an intraperitoneal toxicity study was performed. Six albino Wistar rats were used in the investigation, and they were administered montelukast intraperitoneally at a single dose of 50 mg/kg. The rats which were treated were observed for general clinical signs and symptoms and mortality for a period of 24 hours and up to 14 days. Even at a dosage of 50 mg/kg, montelukast was reported to cause no death in rats. Therefore, montelukast at doses 10 and 20 mg/kg were chosen and deemed safe for additional research.

Grouping of animals

The experimental rats have been classified into four seperate groups having six rats (n=6) in each group. The drugs montelukast and caffeine were dissolved in normal saline (0.9% NaCl). Freshly prepared solutions were administered to the experimental rats daily for a period of 10 days as follows [8]:

• Group 1: vehicle control group received 1ml saline intraperitoneally.

• Group 2: first test group received Montelukast 10mg/kg intraperitoneally.

• Group 3: second test group received Montelukast 20mg/kg intraperitoneally

• Group 4: treatment control group received standard drug Caffeine 50mg/kg intraperitoneally

Evaluation of Anxiety effects

The anxiety related behavior of montelukast was evaluated using Open Field Test (OFT), Elevated Plus Maze (EPM) and Social Interaction Test (SIT) [9] [10]. These behavioural assessments were completed in between 9:00 am to 6:00 pm on the days‘0’and‘10’of the experiment. A week before the trial began, the animals were moved to the testing room for getting habituated to the surroundings. The experimental rats were initially used for OFT and then for EPM test, followed by use in SIT. The washout period in between the experiments using the various apparatus was 7 days [11].

i) Elevated Plus Maze Test:

The black polypropylene elevated plus maze test apparatus was set up in the shape of a ‘+’with a central platform measuring 25cm x 5cm x 0.5 cm and two open arms measuring 25cm x 5cm x 0.5 cm perpendicular to each other and two closed arms measuring 25cm x 5cm x 16 cm. The closed arms had a 16cm wall to support the arm, whereas the open arms had a very small (0.5cm) wall to limit the amount of falls. The whole device was at a height of 50 centimetres above the ground. Each rat was positioned at the intersection of four arms, pointed to an open arm, and allowed five minutes to navigate the maze. The activities of the rat were videotaped using a video camera. This test was carried out on days 0 and 10 of the experiment over a duration of 5 minutes. The parameters used to assess anxiety-like effects in the elevated plus maze are number of times the Open Arm (OAE) and Closed Arm (CAE) were entered, along with the time duration spent in the closed arm (TCA) and the time duration spent in the open arm (TOA). After every test, the maze was thoroughly rinsed with alcohol and water.

ii) *Open Field Test*:

The open field apparatus is 72cm x 72cm in size with 36cm walls, constructed out of white plywood. Using a marker, black lines were painted on the base to create sixteen 18cmx 18cm squares. In the middle of the open field, the central square was drawn. During the test, each rat was positioned in the lower right corner of the open field setup and recorded for five minutes with a video camera kept at a distance of 100cm away. Between experiments, the arena was wiped down using cotton moistened with 70% alcohol. In the open field test, the following parameters were measured for five minutes: central latency (the amount of time taken to get into the open field test's center zone), fecal pellets (number of feces secreted by every single rat), number of lines

crossed, and rearings (standing on their hindlimbs without touching the wall). These measures were used to assess anxiety-like effects. Locomotor activity is often indicated by the quantity of lines crossed and the frequency of rearing. The frequency of central square entries, the amount of time spent in the center square, and the latency to enter the central square are the indicators of anxiety and exploratory behavior. Anxiolysis is indicated by a decline in latency to reach the central part or an increase in the amount of time spent there [12]

iii) *Social Interaction Test*:

The experimental rats were subjected to the social interaction test at two time points: on day 1 (initiation of treatments), day 10 (completion of the study). Prior to each assessment, the animals were housed individually for five consecutive days to minimize prior social influence. The test apparatus consisted of a wooden box measuring 60cm by 60cm by 35cm, positioned in a dimly lit space to reduce stress. On the sixth day, each rat was introduced individually into the apparatus and allowed two familiarisation sessions of 7.5-minute each, separated by two hours interval. The same sexed rats were paired according to their weight and kept in the apparatus for 7.5 minutes on the tenth day. A total of 7.5 minutes was recorded as the time the animal spent in "social interaction," which included sniffing, biting, grooming, boxing, kicking, and crawling under or over each other.

Statistical Analysis:

All the test values are stated as mean± SEM (standard error of mean). The data was statistically analyzed with SPSS software (version 24).The values were examined with the use of one-way analysis of variance (ANOVA) accompanied by a post hoc test, with a p-value of less than 0.05 indicating statistical significance.

3. RESULTS

i) Effect of Montelukast on Elevated Plus Maze

From Table 1,a significant decrease in open arm entries, closed arm entries and time spent in the open arms was observed on day 10 compared to day 1 in rats treated with montelukast (20 mg/kg) and caffeine (50 mg/kg). Time spent in closed arms increased significantly on day 10 as compared to day 1 in both the groups receiving montelukast (20 mg/kg) and caffeine (50 mg/kg) .

ii) Effect of Montelukast on Open Field Test

From Table 2,a significant decrease was observed in the number of lines crossed, rearings, central latency on day 10 as compared to day 1 in the rats receiving montelukast 10mg/kg, 20mg/kg and caffeine 50mg/kg. Fecal pellet has increased significantly on day 10 as compared to day 1 in all the three groups.

iii) Effect of Montelukast on Social interaction Test

From Table 3, it was seen that there was a significant decrease in the SIT on the 10th day of the experiment in the rats receiving montelukast 10mg/kg, 20mg/kg and caffeine 50mg/kg as compared to day 1.

TABLE 1: Effect of Montelukast on Elevated Plus Maze

	DAY 1				**DAY 90**			
(vehicle control)								
(montelukast 10mg/kg)								
GROUP 3 (montelukast 20mg/kg)	3.83±1.12	4.89±1.17	110±11.23	159±19.77	0.83±0.77	1.93±0.13	30±11.56	232±24.44
					*	#	x	y
50mg/kg)					*	#	x	y

OAE - OPEN ARM ENTRIES TOA– TIME SPENT IN OPEN ARM

CAE– CLOSE ARM ENTRIES TCA– TIME SPENT IN CLOSE ARM

The Data is presented as mean± SEM, with a sample size of n=6, and data were analyzed using one-way ANOVA followed by post hoc test. $^{*\#xy}$P value <0.05 compared to Day 1, in corresponding groups receiving the same dose of drugs.

TABLE 2: Effect of Montelukast on Open Field Test

	DAY 1 (5 MINUTES DURATION)				DAY 90 (5 MINUTES DURATION)			
	NUMBER OF LINES CROSSED	REARINGS	CENTRAL LATENCY	FECAL PELLETS	NUMBER OF LINES CROSSED	REARINGS	CENTRAL LATENCY	FECAL PELLET
GROUP 1	43.56±11.23	19.56±0.08	23.12±2.59	0.00	47.52±13.24	21.25±0.12	27.03±1.23	0.00
GROUP 2	39.13±10.59	17.12±0.07	20.29±1.53	0.1	8.59±0.06 *	4.29±0.08 #	3.12±0.09 x	1.2±0.3 y
GROUP 3	36.18±10.23	15.03±0.05	18.19±1.13	0.2	5.27±0.02 *	1.21±0.02 #	1.01±0.02 x	1.7±0.4 y
GROUP 4	32.23±9.59	13.79±0.04	16.23±1.08	0.3	4.52±0.01 *	0.88±0.01 #	0.59±0.01 x	2.0±0.9 y

The Data is presented as mean± SEM, with a sample size of n=6, and data were analyzed using one-way ANOVA followed by post hoc test. $^{*\#xy}$ p value <0.05 compared to Day 1, in corresponding groups receiving the same dose of drugs.

TABLE 3: Effect of Montelukast on Social interaction test

Social Interaction Time (Seconds)			
Groups	**Treatment**	**Day 1**	**Day 10**
Group 1	Control	62.53 ± 0.60	62.33 ±1.38
Group 2	Montelukast 10mg/kg	61.25 ±0.42	15.5 ±1.43^{x}
Group 3	Montelukast 20mg/kg	65.15 ±0.23	12.83 ±1.42^{y}
Group 4	Caffeine 50mg/kg	63.15 ±0.13	10.83 ±1.01^{z}

All values are presented as mean± SEM, n=6, and the data were analysed using a one-way ANOVA test followed by a post-hoc test. On day 10, $^{x, y,z}$ P value < 0.001 when compared to Day 1, in corresponding groups receiving the same dose of drugs.

4. DISCUSSION

The guidelines for the treatment of asthma recommends montelukast, a significant and frequently employed medication. In the current study, experimental rodent models of anxiety were employed to evaluate the anxiety like behavioral effects on montelukast administration. Caffeine, a nonselective antagonist of A1 and A2A adenosine receptors, is among the most commonly consumed legal psychoactive agents worldwide. Studies have reported that lower doses of caffeine can alleviate anxiety and depressive symptoms in humans, whereas higher doses may provoke anxiety. Owing to its anxiogenic properties, caffeine was used as the standard drug in the present study. Earlier investigations suggest that the stimulant actions of low-dose caffeine are mainly mediated through blockade of A2A receptors, while inhibition of A1 receptors may contribute to its depressant effects [12].

The EPM test is widely used for the assessment of the emotional component and psychomotor behavior of rodents. The rodents usually occupy dimly lit areas and generally stay away from well-lit spaces; they also exhibit unrest when placed at heights. In this test, the increased number of entries into the open and closed arm, time spent in the closed arm, are considered as the most reliable indicators for decreased anxiety-like activity of the compound, while anxiogenic drugs show the opposite effects [13]. In the EPM test, montelukast at a dose of 20mg/ kg showed a noticeable decrease in entry of rats in the open and closed arms and increased time spent in the closed arm, demonstrating anxiogenic effect. The OFD test has been widely used for screening the effects of anxiolytic and antidepressant compounds. Rodents prefer to stay in peripheries as compared to activities in the centre. The number of lines a rat crosses and how often it rears up are commonly used indicators of exploration and anxiety. Grooming has been hypothesised to decrease anxiety in stressful situation [12]. In this test, the rats are exposed to an unusual atmosphere where they show anxiety in the form of decreased grooming, rearing, and fewer lines crossed. In our study, we found that montelukast given in 10mg/kg and 20mg/kg dose significantly decreased the frequency of lines crossed, and worsened the rearing and grooming behavior. Anxiogenic drugs reduce the duration of social interaction in a new environment [14]. The test drug montelukast at 10mg/kg, 20mg/kg doses significantly decreased the social interaction time, like that of the treatment control group.

Being leukotriene antagonists, montelukast and zafirlukast bind and block the cysteinyl leukotriene receptor 1 (CysLT1) in the cell membrane [15]. These drugs act by blocking cysteinyl leukotriene receptors, thereby inhibiting the actions of leukotrienes C4, D4, and E4, which are involved in inflammatory cell recruitment, alteration of vascular permeability, impairment of ciliary activity, and bronchoconstriction. Although leukotrienes are present in the brain and central nervous system, their exact physiological function remains unclear [16]. Leukotrienes have a role in inflammation in the brain, just as they do in other tissues. Astrocytes and microglial cells

in the brain generate several inflammatory mediators, including leukotrienes. These mediators, along with components of the complement cascade, play a major role in the development of various neuroinflammatory disorders.[17].Leukotrienes are known to play a role in pain response [18]. Concurrently montelukast and other LMTAs cross the blood-brain barrier and are present in brain tissue in considerable amounts [19].As a neuroprotective agent for traumatic brain damage, montelukast has been investigated,[20] and for the management of focal cerebral ischaemia [21] and migraine headaches [22]. Although no causal explanation has been provided, it has been postulated that the neuropsychiatric adverse effects recorded may be caused by inhibition of leukotriene receptors in the brain [23] .Another theory is when montelukast attaches to the cysLT1 receptor nitric oxides are produced, which are detrimental to brain tissue [24] .Nevertheless, it seems that this mechanism is not vindicated by any objective evidence [25]. Although anxiety and asthma control are linked, it was also noted that leukotriene pathways may be involved in causing anxiety-like behavior. Anxiety and transcription factor cFOS expression were reduced in mice lacking 5-lipooxygenase activating protein, an essential protein in eicosanoid metabolism [26].

The findings of this study point towards possible anxiogenic potential of montelukast. However, we could not provide conclusive evidence through histopathological studies of the related involved tissues neither could we highlight the plausible mechanism of this side effect related to the use of montelukast, these may be considered as limitations of our study.

5. CONCLUSION

There is sufficient information to show the positive impact of montelukast in asthma and other respiratory disorders through its various properties. However, recent research revealed montelukast may be associated with anxiety and other neuropsychiatric disorders. This study intends to explore the anxiogenic effects of montelukast in experimental rats. The results of this study will hopefully demonstrate the anxiogenic role of montelukast in asthma. This will further alert the physicians about this possible side effect of montelukast and warrant monitoring the asthma patients more carefully.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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