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Behavioral assessment of zolpidem and zopiclone using four standard rodent models

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Abstract: **Background**: Insomnia is a prevalent sleep disorder affecting a significant proportion of the global population. Non-benzodiazepine hypnotics such as zolpidem and zopiclone are widely used due to their improved safety profile and receptor selectivity. **Objectives**: The present study aimed to comparatively evaluate the effects of zolpidem and Zopiclone on locomotor and behavioral activities in rodents using standard experimental models. **Methods**: Thirty male Swiss albino rats were divided into three groups (n=10): control (vehicle), Zolpidem (2 mg/kg) and Zopiclone (1 mg/kg). Drugs were administered orally for 15 days. Behavioral assessments including cage crossing, open field activity, forced swimming test and exploratory activity (head dip test) were performed. Data were analyzed using independent t-test, with $p<0.05$ considered significant. **Results**: Zolpidem significantly reduced cage crossing, open field activity, struggling time and exploratory behavior compared to control ($p<0.05$), indicating strong sedative and hypnotic effects. Zopiclone showed comparatively milder effects, with less pronounced reductions in locomotor and exploratory parameters. **Conclusion**: Zolpidem exhibited stronger central nervous system depressant effects compared to Zopiclone, likely due to its higher selectivity for α1 subunits of GABA(A) receptors. These findings support its potent hypnotic profile, although differences in behavioral outcomes should be considered in clinical use.

Keywords: GABA receptors; Insomnia; Locomotor activity; Rodent models; Zolpidem; Zopiclone

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INTRODUCTION

Sleep is a natural physiological state of rest that occurs in humans and other animals. It is characterized by a reduced responsiveness to external stimuli compared with wakefulness, while remaining more readily reversible than conditions such as coma (Tononi *et al*., 2024). Sleep plays a vital role in memory consolidation, learning and the restoration of numerous bodily functions. Despite its importance in maintaining both physical and mental well-being, many individuals experience inadequate sleep or suffer from sleep-related disorders.

Sleep disorders are broadly classified into two categories: dysomnias and parasomnias. Dysomnias are generally considered more severe because they primarily affect the quantity, quality, or timing of sleep. In contrast, parasomnias involve abnormal behaviors or experiences occurring during sleep and are often less serious, although they may progress to more significant conditions if left untreated (Chakrabarty, 2023).

Parasomnias encompass a range of sleep-related phenomena, including sleepwalking, sleep talking, night terrors, nightmares, bruxism (tooth grinding), bedwetting and sexsomnia. Although these conditions are typically not regarded as life-threatening, they may be associated with underlying psychological or psychiatric conditions such as mood disorders, anxiety, panic disorders, depression, or excessive alcohol consumption (Biresaw *et al*., 2025; Malhotra and Avidan, 2012).

Dysomnias refer to disorders that impair normal sleep patterns and are commonly associated with insomnia. They are classified into intrinsic and extrinsic types. Intrinsic dysomnias arise from physiological or pathological conditions within the individual, whereas extrinsic dysomnias result from environmental factors or external circumstances that disrupt sleep (Begum, 2023). In some cases, sleep disturbances may also be linked to conditions beyond an individual's control, such as cardiac rhythm abnormalities. Common disorders within the dysomnia category include insomnia, narcolepsy and sleep apnea (Poon *et al*., 2021).

Insomnia is the most common sleep and psychiatric disorder, affecting over 30% of people in industrialised countries (Benjafield *et al*., 2025). It negatively impacts both physical and mental health and is linked to neurodegenerative diseases, depression, anxiety and an increased risk of suicide (Calero and Galiano, 2022). Because sleep is vital for brain homeostasis, plasticity and overall well-being, disturbances in sleep architecture can act as a stressor that worsens mental and physical conditions. Therefore, assessing and treating insomnia is essential. Effective treatments should target the sleep-regulating system and help restore normal sleep architecture, which may also support better stress and emotional regulation(Palagini and Bianchini,

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2022).Several classes of drugs can serve as hypnotic agents, including benzodiazepines, non-benzodiazepines, certain antidepressants, atypical antipsychotics and antihistamines. Among these, benzodiazepines have traditionally been the most widely prescribed for insomnia. However, in recent years, newer non-benzodiazepine hypnotics such as zolpidem and zopiclone have gained prominence due to their favourable safety profile and more selective action on sleep-related GABA receptors. In the present study, we focus specifically on this newer generation of hypnotics to highlight their therapeutic relevance in managing chronic insomnia.

Zolpidem is a prescription medication used for the short-term treatment of insomnia due to its myorelaxant and anticonvulsant properties but very strong hypnotic properties(Maxsumov *et al*., 2024). It is a short-acting with a half-life of 2–3 hours that potentiates gamma-aminobutyric acid GABA and produce sleep within 15 minutes of administration. The underlying mechanism is its selective binding to α1-containing GABAA receptors, which are preferentially found in the brain. Thus, Zolpidem increases slow wave sleep and causes no effect on stage 2 sleep in laboratory test(Palagini and Bianchini, 2022).

Zopiclone is indicated for the short-term treatment of insomnia and is used as a hypnotic, anxiolytic, anticonvulsant and as a myorelaxant. Zopiclone acts indiscriminately at the benzodiazepine binding site on α_1, α_2, α_3 and α_5 GABAA-containing receptors as a full agonist, causing an enhancement of the actions of GABA to produce the therapeutic as well as adverse effects of Zopiclone (Gavat, 2024) Like benzodiazepines, zopiclone and its active metabolite desmethylzopiclone also inhibit N-methyl-D-aspartate (NMDA) receptors and nicotinic acetylcholine (nAChRs) receptors, which might play a role in the addictive properties of these. Zolpidem, an α_1-selective compound while Zopiclone is less selective to the α_1 site and has higher affinity to the α_2 site (Kaygisiz *et al*., 2025).

The new generation hypnotic drugs, zolpidem and zopiclone, are safer options in the treatment of insomnia. The present study is designed for the assessment of cognitive impairment in Non benzodiazepines having hypnotic and sedative activities similar to benzodiazepines. The activity was noted against control for their effects on cage crossing, open field activity, forced swimming and exploratory activity.

MATERIALS AND METHODS

Animals

Thirty Swiss Albino rats were procured from the animal house of the HEJ Research Institute of Chemistry and all were male. The rats weighed between 150 and 200 g and were used in the experiments. The animals were housed in standard-sized polypropylene cages, maintained in a reverse light-dark cycle of 12 hours each and fed a standard diet. Water was available ad libitum. The study was approved by the Advanced Studies and Research Board, University of Karachi and the animals were handled as per the standard guidelines for animal care.

Drugs

Zolpidem and zopiclone were provided by a reputed pharmaceutical company.

Study design

One group consisted of 10 rats and was given Zolpidem at a dose of 2 mg daily and another group of 10 rats was given zopiclone at a dose of 1mg daily. Control rats were given saline daily. As the drugs were given in the doses of 2 mg and 1 mg for zolpidem and zopiclone, respectively (Martín-López and Navarro, 2002) The drugs were powdered with the help of a mortar and pestle. Both drugs were dissolved separately in distilled water. The calculated amount of the solution was administered to the animals orally using a standard laboratory device. Drugs were administered orally for four weeks, once a day.

Experimental parameters

For each animal, the following observations and behavioural tests were conducted.

a) Cage crossing: Animals were placed in the cage and allowed to move freely, four and the number of crossings by the rat in all four directions of the cage was noted (Marques *et al*., 2008). This activity was repeated on alternate days and was finally observed after 28 days of drug administration.

b) Open field: The open field apparatus consists of a square area measuring 76 x 76 cm with walls 42 cm high. The floor was divided into 25 equal squares by lines. Each rat was kept in the centre of the open field (Gould *et al*., 2009). Behavioral changes were assessed exactly after 30 minutes after oral administration of the drug. The test was repeated on alternate days and finally observed after 28 days of administration.

c) Forced swimming test: Rats were forced to swim in a water tank; the water level should be ¾ of the tank. Rats are not habituated to swimming, so it starts struggling to escape for 2-3 minutes, then the rat stops struggling and starts swimming (Petit-Demouliere *et al*., 2005). The duration during which rats were struggling is called the struggling time. The test was repeated on alternate days and observed finally after 28 days of administration.

d) Exploratory activity (head dip): Rats were placed in a box having 16 equally spaced holes. Rats have an inquisitive nature (Takeda *et al*., 1998). They try to explore by dipping their head in the holes. The number of head dips was noted for 10 minutes for each rat. The rat's activity was assessed by head-dip scores. The test was repeated on alternate days and observed finally after 28 days of administration.

Table 1: Observation on gross behavior (Zolpidem).

Parameters	Control	Zolpidem treated			
Grooming	Normal	No change	-	-	-
Twitches	-	-	-	-	-
Ptosis	-	+	+	+	+
Hind paw abduction	-	-	+	+	+
Facial passivity	-	-	+	+	+
Pinna reflex	-	+	+	+	+
Corneal reflex	-	+	+	+	+
Exopthalamus	-	-	-	-	-
Anopthalamus	-	-	-	-	-
Salivation	Normal	No change	No change	No change	No change
Lacrimation	Normal	No change	No change	No change	No change
Defecation	Normal	No change	No change	No change	No change
Urination	Normal	No change	No change	No change	No change
↓ Motor activity	Normal	+	+	+	++

e) General observations: All the animals were observed for acute behavioral changes of the drugs. These include grooming, ptosis, hind paw abduction, facial passivity , tremor, twitches, righting reflex , pinna reflex, corneal reflex, exopthalamus, anapthalamus, salivation, lacrimation, defecation and urination etc (Moser, 1999).

Statistical analysis

The statistical analysis was carried out by SPSS ver 19. The values were represented as Mean ± S.D. Significant differences were analyzed by independent t-test ($p<0.05$).

RESULTS

General observations

In the present study, a variety of acute behavioral changes were observed like ptosis, facial passivity, hind paw abduction, decreased motor activity, drowsiness etc. Corneal and pinna reflexes were also affected. Urination, defecation and salivation were normal.

Cage crossing

The Cage crossing activity score by zolpidem-treated rats when compared to that of the saline-treated control group. The results are significantly different when analyzed using a t-test (Table 3). Cage crossing activity score by zopiclone-treated rats when compared to that of the saline-treated control group was not significantly different when analyzed by t-Test (Table 3). The results indicate that zolpidem produced a greater reduction in cage crossing than zopiclone.

Open field activity

The Open field activity score by zolpidem-treated rats when compared to that of the saline-treated control group was significantly different when analyzed by t-Test (Table 3). While the open field activity score by the zopiclone-treated control group was not significantly different when analyzed by t-Test (Table 3). The reduction in Open field activity observed with zolpidem was more significant than with zopiclone.

Forced swimming

In the forced swimming test, the Struggling time in zolpidem-treated rats was significantly different from that of the saline-treated group**.** Struggling time noted in the zopiclone-treated rats was also significantly different from that of the saline-treated rats when analyzed by t-test (Table 3). zopiclone produced a more significant reduction in struggling time than zolpidem.

Head dip (exploratory activity)

Exploratory activity noted by zolpidem-treated rats was significantly different when compared to that of the saline-treated control group when analyzed by t-Test (Table 3). While the exploratory activity score of zopiclone-treated rats was not significantly different from that of the vehicle-treated control group, the reduction in exploratory activity observed with zolpidem was more significant than with zopiclone.

DISCUSSION

The present study evaluated and compared the behavioral and locomotor effects of zolpidem and zopiclone using established rodent models. Both drugs produced a reduction in locomotor and exploratory activities, indicating central nervous system (CNS) depressant effects; however, zolpidem exhibited a more pronounced effect across all parameters (Kaygisiz *et al*., 2025).

The significant reduction in cage-crossing and open-field activity following zolpidem administration suggests strong sedative and hypnotic properties. This effect can be attributed to its selective binding to the ω1 (BZ1) site of the GABA(A) receptor, particularly the α1 subunit, which is primarily associated with sedation and sleep induction.

Table 2: Observation on gross behavior (Zopiclone).

Parameters	Control	Treated (Zopiclone)				
Grooming	Normal	No change	No change	-	-	-
Twitches	-	-	-	-	-	-
Ptosis	-	-	+	+	+	++
Hind paw abduction	-	-	+	+	+	+
Facial passivity	-	-	+	+	+	++
Pinna reflex	-	-	+	+	+	++
Corneal reflex	-	-	+	+	+	+
Exopthalamus	-	-	-	-	-	-
Anopthalamus	-	-	-	-	-	-
Salivation	Normal	No change	No change	No change	No change	No change
Lacrimation	Normal	No change	No change	No change	No change	No change
Defecation	Normal	No change	No change	No change	No change	No change
Urination	Normal	No change	No change	No change	No change	No change
↓ Motor activity	Normal	-	-	+	++	++

Table 3: Effect of drugs on behavioral parameters.

Groups	N	Behavioral parameters			
		Cage crossing Mean ± S.D	Open field Mean ± S.D	Forced swimming (Struggling time sec)	Head dip Mean ± S.D
Control (saline)	10	17.6±3.4	29.6±3.32	82.7±31.9	110.4±55.42
Zolpidem	10	6.7±3.12*	25.1±3.7*	50.6±32.0	66.1±26.83*
Zopiclone	10	11.0±3.6	29.2±3.9	36.6±25.9*	110.0±37.5

Values are means ± S.E Significant differences analyzed by independent t-test ($p<0.05$). *Results are significantly different ($P<0.05$) from saline-treated animals

In contrast, zopiclone interacts less selectively with multiple GABA(A) receptor subunits (α1, α2, α3 and α5), resulting in comparatively milder sedative effects (Richter *et al*., 2020).

The reduction in time spent struggling in the forced swimming test further supports the CNS-depressant action of zolpidem. This may be associated with its inhibitory effects on neuronal excitability in motor regulatory pathways, including basal ganglia circuits. Zopiclone also reduced struggling time (Hossen *et al*., 2022).

The exploratory behavior observed through the head dip test revealed a marked decrease in activity following zolpidem administration, suggesting reduced alertness and increased sedation(Choo *et al*., 2026) In contrast, zopiclone-treated animals showed minimal changes, indicating preservation of certain behavioral functions(Najam *et al*., 2011) This difference may be due to zopiclone's relatively lower selectivity for sedative-specific receptor subtypes.

All results highlighted that zolpidem exerts greater suppression of locomotor and exploratory behaviour compared to zopiclone, supporting its potent sedative profile.

CONCLUSION

The present study demonstrates that both zolpidem and zopiclone exhibit central nervous system depressant effects, as evidenced by reduced locomotor and exploratory activities in rodents. However, zolpidem produced significantly stronger effects compared to zopiclone, likely due to its selective action on α1-containing GABA (A) receptors. These findings suggest that zolpidem may have a more potent sedative profile, whereas zopiclone may preserve certain behavioral functions. Further studies are required to explore the underlying molecular mechanisms.

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Author's contributions
Hina Ilyas: Conceptualization, formal analysis, investigation, writing – original draft. Memoona Gillani: Resources

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Data availability statement
The datasets generated and evaluated during this study will be available from the corresponding authors on request.

Ethical approval
All the protocols of the experiments were adhered to the ethical guidelines for the care and use of laboratory animals, approved by the Institutional Bioethical Committee (Approval No.: IBC KU-12486/2007).

Conflict of interest

There is no conflict of interest.

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