## Abstract

**Introduction/aim:** Diazepam is commonly used in clinical setting in treatment and management of several conditions such as convulsion, insomnia, anxiety disorder and sleep disorder. Caffeine is widely and regularly consumed for different purposes. It is a central nervous system stimulant that affects the body in numerous ways. The aim of this study is to investigate the effect of caffeine on diazepam– induced in rat.

**Method:** A total of thirty (30) wister rats of 120–210 g of either sex were divided into five groups of six mice per group. Rats in all group received diazepam (4 mg/Kg), while group 2, 3, 4 and 5 received concurrent dose of caffeine (2.5, 5, 10 and 20 mg/Kg) intraperitoneally respectively. After 2 minutes of administration of the drugs, sedative and hypnotic study were carried out.

**Result:** There was significant ($P<0.05$) dose dependent decreased in the time taken for rat in all groups to return the widely parted hind limb to their normal position when compare to the control. There was also significant ($P<0.05$) dose dependent increased in sleep latency and decreased in duration of sleep in all group administered caffeine. Group 5 rats did not have sleep latency and duration of sleep throughout the 90 minutes period of observation.

**Conclusion:** result from the study showed that caffeine significantly reduced CNS effect of diazepam induced rats which suggests that dose adjustment should be considered to patients on diazepam who may have been exposed to caffeine.

**Keyword:** diazepam, caffeine, rats, sedative, hypnotic

## Introduction

Sedatives and hypnotics are the drugs which can reduce anxiety and produce a calming effect by inducing the onset of sleep as well as maintaining sleeping duration[1].

Benzodiazepines (BZDs) act as positive allosteric modulators on the gamma amino butyric acid (GABA)-A receptor. The GABA-A receptor is a ligand-gated chloride-selective ion channel. GABA is the most common neurotransmitter in the central nervous system, found in high concentrations in the cortex and limbic system. GABA is inhibitory in nature and thus reduces the excitability of neurons. GABA produces a calming effect on the brain.[2] The 3 GABA receptors are designated A, B, and C. Diazepam is mainly used to treat anxiety, insomnia, panic attacks and symptoms of acute alcohol

withdrawal[3]. It is also used as a premedication for inducing sedation, anxiolysis, or amnesia before certain medical procedures (e.g., endoscopy).[4] In 2020, it was approved for use in the United States as a nasal spray to interrupt seizure activity in people with epilepsy.[5,6] Diazepam is the drug of choice for treating benzodiazepine dependence with its long half-life allowing easier dose reduction. Benzodiazepines have a relatively low toxicity in overdose[7]. Adverse effects of benzodiazepines such as diazepam include anterograde amnesia, confusion (especially pronounced in higher doses) and sedation. The elderly are more prone to adverse effects of diazepam, such as confusion, amnesia, ataxia, and hangover effects, as well as falls. Long-term use of benzodiazepines such as diazepam is associated with drug tolerance, benzodiazepine dependence, and benzodiazepine withdrawal syndrome.[8] Like other benzodiazepines, diazepam can impair short-term memory and learning of new information.[9]

Caffeine is a psychostimulant with the same central effects as the classical nervous system psychostimulants cocaine and amphetamine, according to Sergi Ferré[10]. That is, it increases motor activity and has both arousal and reinforcing effects, although its reinforcing effects are not as strong as those of the classical psychostimulants[11]. Caffeine is a central nervous system stimulant that reduces fatigue and drowsiness.[12] At normal doses, caffeine has variable effects on learning and memory, but it generally improves reaction time, wakefulness, concentration, and motor coordination.[13,14] The amount of caffeine needed to produce these effects varies from person to person, depending on body size and degree of tolerance.[15,16,17] The desired effects arise approximately one hour after consumption, and the desired effects of a moderate dose usually subside after about three or four hours.[18]

Caffeine is an antagonist of adenosine $A_{2A}$ receptors, and knockout mouse studies have specifically implicated antagonism of the $A_{2A}$ receptor as responsible for the wakefulness-promoting effects of caffeine.[19]Antagonism of A2A receptors in the ventrolateral preoptic area (VLPO) reduces inhibitory GABAneurotransmission to the tuberomammillary nucleus, a histaminergic projection nucleus that activation-dependently promotes arousal.[19] This disinhibition of the tuberomammillary nucleus is the downstream mechanism by which caffeine produces wakefulness-promoting effects.[19] Caffeine is an antagonist of all four adenosine receptor subtypes ($A_1$, $A_{2A}$, $A_{2B}$, and $A_3$), although with varying potencies.[20,21] The affinity ($K_D$) values of caffeine for the human adenosine receptors are 12 μM at $A_1$, 2.4 μM at $A_{2A}$, 13 μM at $A_{2B}$, and 80 μM at $A_3$.[22]

Caffeine can delay or prevent sleep and improves task performance during sleep deprivation.[23] Shift workers who use caffeine make fewer mistakes due to drowsiness.[39] Caffeine overdose can result in a state of central nervous system over-stimulation known as caffeine intoxication, a clinically significant temporary condition that develops during, or shortly after, the consumption of caffeine.[24] This syndrome typically occurs only after ingestion of large amounts of caffeine, well over the amounts found in typical caffeinated beverages and caffeine tablets (e.g., more than 400–500 mg at a time). According to the DSM-5, caffeine intoxication may be diagnosed if five (or more) of the following symptoms develop after recent consumption of caffeine: restlessness, nervousness, excitement, insomnia, flushed face, diuresis (increased production of urine), gastrointestinal disturbance, muscle twitching, rambling flow of thought and speech, tachycardia (increased heart rate) or cardiac arrythmia, periods of inexhaustibility, and psychomotor agitation.[25,26]The aim of this study is to determine the dose dependent effect of caffeine on diazepam induced mice.

## Materials and method

### Animals

Male and female wister rats were obtained from Bingham University, Animal House. They were maintained on standard animal pellets and given water ad libitum. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee of Bingham University.

### Hind-limb parting test

A total of thirty (30) wister rats of 120–210 g of either sex were divided into five groups of six mice per group. Rats in all group received diazepam (4 mg/Kg), while group 2, 3, 4 and 5 received a concurrent dose of caffeine (2.5, 5, 10 and 20 mg/Kg) respectively intraperitoneally. After 2 minutes of administration of the drugs the hind limb of each rat was widely parted and the time taken for the limbs to return to its normal position was noted.

### Diazepam-Induced Sleeping Time Determination

The sleep latency (time between diazepam administration and loss of righting reflex) and duration of sleep (from loss of righting reflex to recovery of the reflex) were recorded for each animal. The control animals were treated with diazepam alone. Group 1 diazepam (4 mg/Kg) served as the control.

**Statistical analysis**

Data were expressed as the Mean ±Standard Error of the Mean (SEM). Data were analyzed statistically using one-way Analysis of Variance (ANOVA) followed by Dennett's post hoc test for multiple comparisons between the control and treated groups. Values of $P \leq 0.05$ were considered significant.

**Result**

**Effect of caffeine on diazepam induced sedation in rat**

There was significant ($P<0.05$) dose dependent decrease in the time taken for rat in all groups to return their widely parted hind limb to normal position when compare to the control.

**Table 1: Effect of caffeine on diazepam induced sedation in rat**

| S/N | Group | Dose | Sedative effects (sec) |
|---|---|---|---|
| 1 | Group 1 | Diazepam 4 mg/kg | 15.0±0.5 |
| 2 | Group 2 | Diazepam 4 mg/kg + Caffeine 2.5 mg/kg | 4.15±0.2* |
| 3 | Group 3 | Diazepam 4 mg/kg + Caffeine 5 mg/kg | 4.00±0.2* |
| 4 | Group 5 | Diazepam 4 mg/kg + Caffeine 10 mg/kg | 3.15±0.3* |
| 5 | Group 6 | Diazepam 4 mg/kg + Caffeine 20 mg/kg | 2.90±0.4* |

*significantly different from the diazepam alone adminstered control at $p < 0.05$.

**Effect of caffeine on diazepam induced rat on sleep latency**

There was significant ($P<0.05$) dose dependent increase in sleep latency in all group administered caffeine. Group 5 rats did not have sleep latency throughout the 90 minutes period of observation.

**Table 2: Effect of caffeine on diazepam induced rat on sleep latency**

| S/N | Group | Dose | Sleep latency (seconds) |
|---|---|---|---|
| 1 | Group 1 | Diaz 4 mg/kg | 67.0±0.5 |
| 2 | Group 2 | Diaz 4 mg/kg + Caf 2.5 mg/kg | 360±3.5* |
| 3 | Group 3 | Diaz 4 mg/kg + Caf 5 mg/kg | 450±5.4* |
| 4 | Group 5 | Diaz 4 mg/kg + Caf 10 mg/kg | 631±12.3* |
| 5 | Group 6 | Diaz 4 mg/kg + Caff 20 mg/kg | >3600* |

*significantly different from the diazepam alone adminstered control at $p < 0.05$.

**Effect of caffeine on diazepam induced rat on duration of sleep**

There was significant ($P<0.05$) dose dependent increase in decrease in duration of sleep in all group administered caffeine. Also, in group 5 rats did not show duration of sleep throughout the period of this study

**Table 3: Effect of caffeine on diazepam induced rat on duration of sleep**

| S/N | Group | Dose | Duration of sleep (seconds) |
|---|---|---|---|
| 1 | Group 1 | Diaz 4 mg/kg | 1200±12.5 |
| 2 | Group 2 | Diaz 4 mg/kg + Caf 2.5 | 420±25.4* |

| | | | |
|---|---|---|---|
| | | mg/kg | |
| 3 | Group 3 | Diaz 4 mg/kg + Caf 5 mg/kg | 330±30.32* |
| 4 | Group 5 | Diaz 4 mg/kg + Caf 10 mg/kg | 51±13.6* |
| 5 | Group 6 | Diaz 4 mg/kg + Caff 20 mg/kg | 0* |

*significantly different from the diazepam alone adminstered control at $p < 0.05$.

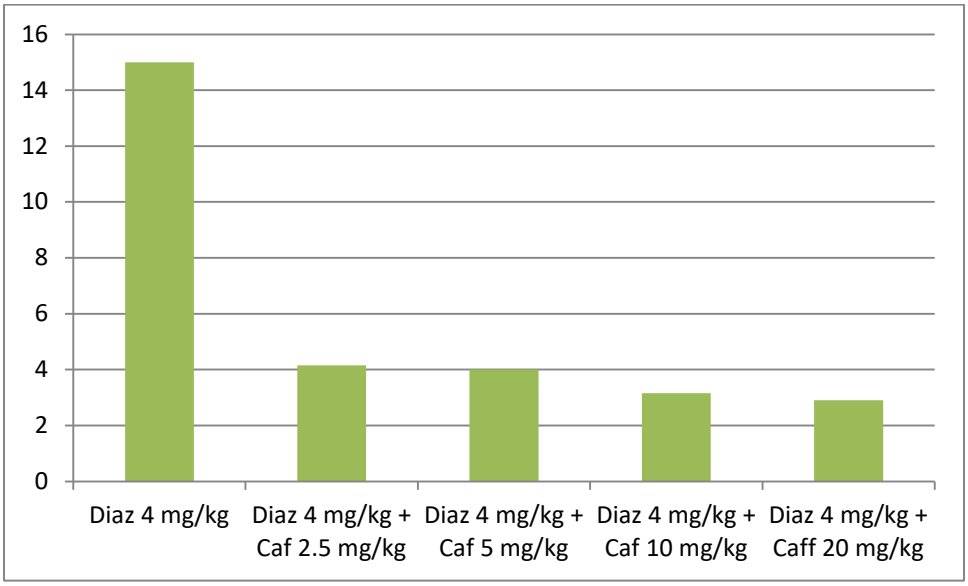


**Figure 1: Effect of caffeine on diazepam induced sedation**

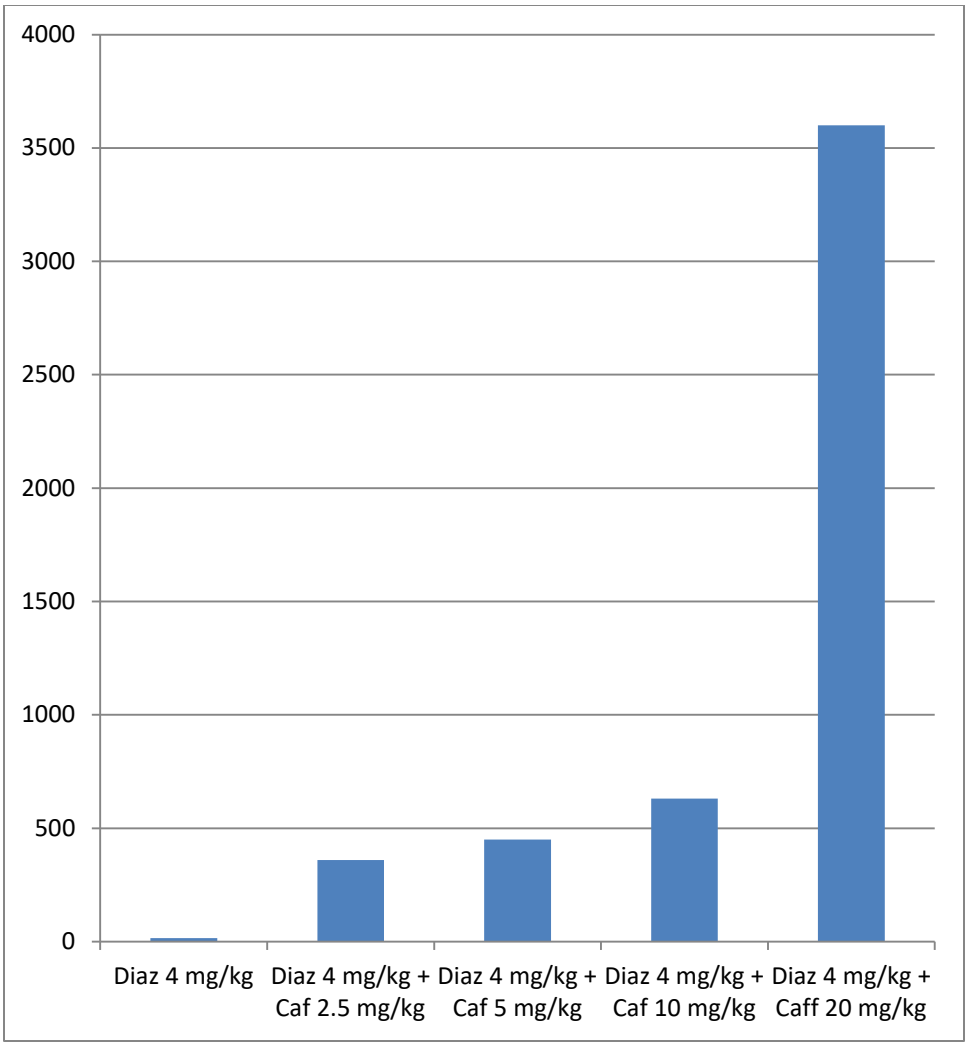


**Figure 2: Effect of caffeine on diazepam induced sleep latency**

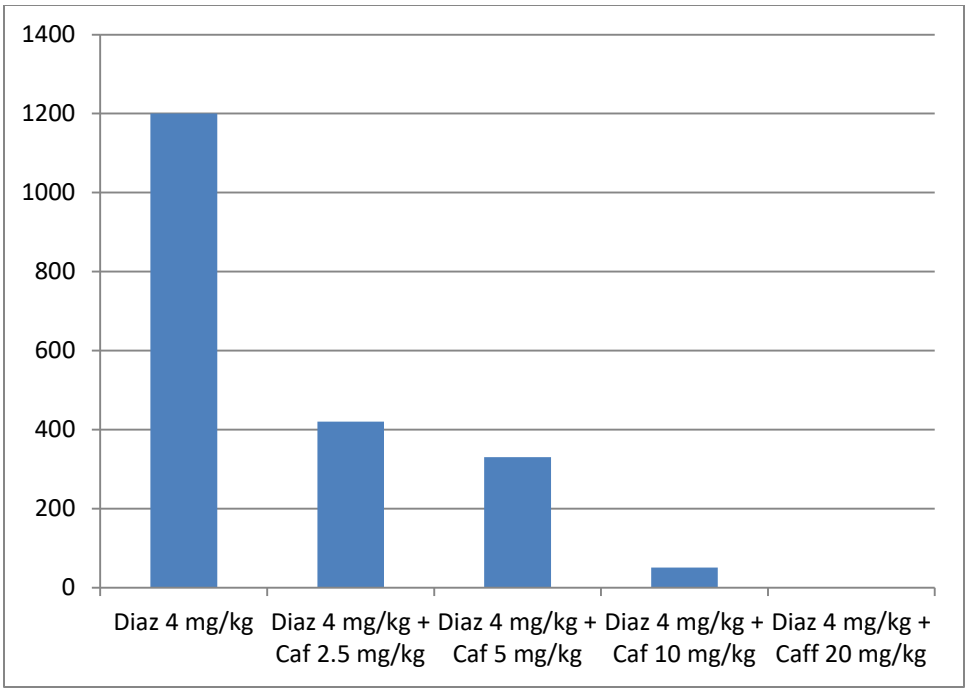


**Figure 3: Effect of caffeine on diazepam induced duration of sleep**

## Discussion

Drugs are chemical substances intended to correct or maintain the body physiological state or function. Drug interaction can affect the pharmacokinetic and pharmadynamic outcome of a particular drug[27,28]. In this era of clinical polypharmacy, it is imperative to understand the possible pharmacological outcome, whether synergistic, antagonistic or no pharmacological consequence which may result from this combination[29,30,31]. In this study caffeine effectively and dose dependently reduced the sedative effect in the diazepam induced rat. Also, there was significant reduction in sleep latency time and observable decrease in duration of sleep in all groups that received caffeine. In fact, at the highest dose administered there was little to no hypnotic effect observed.

Diazepam is a benzodiazepine tranquilliser with anticonvulsant, sedative, muscle relaxant and amnesic properties [16,32].Benzodiazepines, such as diazepam, bind to receptors in various regions of the brain and spinal cord. This binding increases the inhibitory effects of gamma-aminobutyric acid (GABA) [9]. GABAs functions include CNS involvement in sleep induction. Also involved in the control of hypnosis, memory, anxiety, epilepsy and neuronal excitability. Benzodiazepines are positive allosteric

modulators of the GABA type A receptors ($GABA_A$). The $GABA_A$ receptors are ligand-gated chloride-selective ion channels that are activated by GABA, the major inhibitory neurotransmitter in the brain[33]. Binding of benzodiazepines to this receptor complex promotes the binding of GABA, which in turn increases the total conduction of chloride ions across the neuronal cell membrane. This increased chloride ion influx hyperpolarizes the neuron's membrane potential[34]. As a result, the difference between resting potential and threshold potential is increased and firing is less likely. As a result, the arousal of the cortical and limbic systems in the central nervous system is reduced.[35]

Caffeine is the most widely used central nervous system (CNS) stimulant in the world[36]. It has numerous pharmacological and physiological effects, including cardiovascular, respiratory, renal, and smooth muscle effects, as well as effects on mood, memory, alertness, and physical and cognitive performance[37]. Caffeine is an antagonist of adenosine $A_{2A}$ receptors, and knockout mouse studies have specifically implicated antagonism of the $A_{2A}$ receptor as responsible for the wakefulness-promoting effects of caffeine.[38]Antagonism of A2A receptors in the ventrolateral preoptic area (VLPO) reduces inhibitory GABAneurotransmission to the tuberomammillary nucleus, a histaminergic projection nucleus that activation-dependently promotes arousal.[39,40] This disinhibition of the tuberomammillary nucleus is the downstream mechanism by which caffeine produces wakefulness-promoting effects.[41] Caffeine is an antagonist of all four adenosine receptor subtypes ($A_1$, $A_{2A}$, $A_{2B}$, and $A_3$), although with varying potencies.[42] The affinity ($K_D$) values of caffeine for the human adenosine receptors are 12 μM at $A_1$, 2.4 μM at $A_{2A}$, 13 μM at $A_{2B}$, and 80 μM at $A_3$.[37,42]

Some studies[43,44,45,46] have shown that caffeine modifies or antagonizes the effects of benzodiazepines on behavior in both animals and humans. The mechanism for this antagonism was proposed to be the blocking of benzodiazepine receptors by caffeine. Caffeine does have weak antagonistic properties at these receptors. However, this mechanism requires very high concentrations of caffeine[48,49]. Another studies[50,51] suggested that the interaction between caffeine and benzodiazepines is mediated through caffeine's effects on adenosine receptors. There is some evidence that caffeine may also be a histamine receptor antagonist[52].

Caffeine caused increase in sedation effect, sleep latency time and decrease in sleep duration in the diazepam induced rats. This may be due to effect on $GABA_A$ receptors ligand-gated chloride-selective ion channels that are activated by GABA. Indirect or direct effect of caffeine on this receptor complex

may reduces or prevents the binding of GABA, which in turn may decreases the total conduction of chloride ions across the neuronal cell membrane. The study also showed that at much higher dose the hypnotic effect may be completely lost. This may be due to the ability of caffeine to completely antagonize the effect of diazepam on GABA and adenosine $A_{2A}$ receptors.

**Conclusion:** caffeine significantly affects the sedative and hypnotic activity of diazepam. This is of immense pharmacological and clinical implication. There may be need to proportionately consider adjusting dose of diazepam to patient with condition related to central nervous system who have been exposed to caffeine. Considering the fact that caffeine is regularly consumed worldwide, more study may be necessary to determine the effect of caffeine on other CNS acting drugs and other health conditions.

**Conflict of Interest**

The authors declare that there are not any potential conflicts of interest.

**Acknowledgement**

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