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QUANTITATIVE ASSESSMENT AND TIME KINETICS OF ZOPICLONE (THE MOST USED HYPNOTIC DRUG) IN THE STORED BLOOD SAMPLES

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ABSTRACT

Background: Zopiclone is a hypnotic short-acting agent used in the treatment of primary insomnia. After oral administration, zopiclone is rapidly absorbed and has a bioavailability of about 80%. Zopiclone is metabolized extensively in the liver, but the CYP isoforms involved in its metabolism have not yet been identified. **Objective:** To estimate the zopiclone levels in the drawn plasma of volunteers at different time points after storage under refrigeration. To collect data about the insomnia or sleepiness after 24hours of single oral dose of zopiclone. **Methods:** There were totally 42 volunteers belonging to the age groups 22-44 years, 45-60 years and 61-76 years group. Drug administration: The volunteers were given an oral dose of zopiclone (5mg) tablet and blood collected at various intervals as follows: 1- After administration, blood samples were collected at 0, 1, 2, 3, 4, 6, 12, 18, 24, 48h and plasma separated for zopiclone estimation. 2- After administration, after 2 hours the blood was collected and plasma separated, which was stored in refrigerator for analysis at various intervals to see the stability of the zopiclone. 3- Urinary levels of Zopiclone was also measured in all the groups. **Results:** In our study, we demonstrated that following the single administration of oral tablet of Zopiclone, the plasma levels diminish very slowly taking upto 48 hours. Some traces of Zopiclone were identifiable upto 72 hours (not presented here). The major difference was the relapse of insomnia very fast among the elderly population and middle aged groups in comparison to the younger group of 22-44 years. In addition, the storage of plasma even under refrigeration resulted in fast degradation of zopiclone in the samples. This suggests strongly that, the zopiclone should be estimated as the sample is fresh. In addition the zopiclone values from stored samples need verification with other assays. **Conclusion:** Z-drugs have few distinct advantages over their predecessors, the benzodiazepines, and in many ways they have similar adverse and toxic effects, especially zopiclone. The effects of Z-drugs largely derive from their GABA ergic action and pharmacokinetic profiles, which decide the extent of efficacy and toxicity. Adverse Z-drug effects and toxicity are more likely with poly drug use in therapeutics and co-ingested psychoactive substances in overdose.

KEYWORDS: Insomnia, Zopiclone.

INTRODUCTION

This review addresses insomnia disorder (ID), by far the most common sleep disorder, as well as the second most common neuropsychiatric disorder, only outnumbered by the Diagnostic and Statistical Manual of Mental Disorders comprehensive category of all anxiety disorders.[1] Insomnia is a subjective experience of poor or unrefreshing sleep that may be apparent from a delayed onset or decreased duration of sleep. Insomnia is an underrecognized and undertreated medical condition that leads to lifestyle impairment, loss of occupational productivity, and potential physical harm from accidents as well as exacerbation of other medical conditions. The rate of diagnosed insomnia in the UK and North America is estimated at 5–15 %, with up to 40 % of the population experiencing symptoms of daytime sleepiness. Some studies quote that up to a third of elderly North Americans are prescribed either a Z-drug or benzodiazepine for sleep disturbance, an alarming statistic given the risks associated with hypnotics in the elderly.[2] Commercially available, non-benzodiazepine drugs in the USA for the treatment of insomnia: zaleplon, zolpidem, and eszopiclone (the active enantiomer of zopiclone). The ideal anti-insomnia drug is a potent sedative during the night without causing the same residual sedation during the daytime. Suboptimal clinical and adverse effects of traditional benzodiazepines have driven the development of alternative sedative–hypnotic drugs. While hypnosis and sedation are adequately achieved from oral benzodiazepines, they invariably alter sleep architecture, reduce deep (stage 3 and 4) sleep, and lead to dependence, tolerance, and withdrawal. Furthermore, benzodiazepines carry the risk of residual daytime effects

such as impairment of cognitive and psychomotor function. Like benzodiazepines, the newer Z-drugs are agonists at the same γ-aminobutyric acid-type A (GABAA) receptor. However, they possess shorter duration of action and half-life, do not disturb overall sleep architecture, and cause less residual effects during daytime hours, making them more clinically attractive than benzodiazepines.[3] Zopiclone is a hypnotic short-acting agent whose chemical structure corresponds to a cyclopyrrolone derivative, which, although not chemically related to existing hypnotics, presents a pharmacologic profile similar to the Benzodiazepines group by binding with high affinity to the pharmacological receptors of them. Zopiclone will often work well in the short term, but it is not normally prescribed for more than two to four weeks. This is because the body gets used to it within a short period of time and after this it is unlikely to have the same effect. The system may also become dependent on it when it is taken for longer periods of time than this. Zopiclone, like the other Z-drugs, zolpidem and zaleplon, interacts with the same molecular target as the benzodiazepines on the GABAA (γ-aminobutyric acid) receptor. Zopiclone is available as the racemic mixture although the S-enantiomer, eszopiclone, has a 50 times higher affinity for the receptor than the R-enantiomer. In terms of clinical efficacy and adverse reactions, the Z-drugs showed little difference from the short acting hypnotic benzodiazepines although the fatality toxicity index (deaths/10^6 prescriptions) for zopiclone, 2.1, was much lower than the 9.9 for temazepam.[4] The Hypnotic mechanisms of Zopiclone GABAA receptors mediate inhibitory synaptic transmission in the central nervous system and are the targets of neuroactive drugs used in the treatment of insomnia.[5] GABAA receptors are pentametric membrane proteins that operate as GABA (γ-aminobutyric acid) ligand-gated chloride channels. Agonists increase the chloride permeability, hyperpolarize the neurons, and reduce the excitability. The receptors are made up of seven different classes of subunits with multiple variants (α1– α6, β1–β3, γ1–γ3, ρ1–ρ3, δ, ε and θ) that are differentially expressed throughout the brain. Most GABAA receptors are composed ofα-, β- and γ-subunits. . ZOP has a high affinity for the benzodiazepine binding site and acts at γ2-, γ3-bearing GABAA receptors, including α1β2γ2 and α1β2γ3, but relative to benzodiazepines, produce comparable anxiolytic effects with less sedation, muscle relaxation, or addictive potential.[5]

After oral administration of the racemic drug, ZOPICLONE is rapidly absorbed from the gastrointestinal tract, with a bioavailability of approximately 80%. Plasma protein binding of ZOPICLONE was reported as 45% in one study and 80% in another. Both albumin and α-1-acid glycoprotein contribute to protein binding but also other plasma proteins might be involved (e.g. globulins, lipoproteins). It has been noticed that the protein binding is stereo selectivity.[6] reported the first large series of zolpidem poisoning cases in 1994, where the toxicity predominantly involved sedation with ingestions up to 1.4g. Rarely did zolpidem cause coma, respiratory depression, cardiovascular toxicity, or death. Since then, reports of agitation, hallucinations, psychosis, and coma from Z-drug overdose have been published. Other unusual reports include hemolytic anemia and methemoglobinemia from zopiclone. Early clinical trials failed to show major morbidity or mortality from Z-drugs either used therapeutically or in overdose. Over the past 15 years, increasing red flags from forensic cases, drug-facilitated crimes, and motor vehicle crash statistics indicate that mortality from Z-drugs may be similar to benzodiazepines. Bizarre behavior, falls, accidents, and other injuries may also lead to death. In the study by Garnier et al.[7] Stability of Zopiclone in the biological samples for future analysis In Sweden specimens of venous whole blood are taken by a nurse or physician, urine samples are collected by the police officers and post-mortem samples (e.g. femoral blood, urine, vitreous humor, hair, liver, brain, kidney and lung) are taken by forensic pathologists. After sampling all specimens are sent to one central laboratory for toxicological analysis. During the transport the samples are stored at ambient temperature for a period of about 20–24 h. However, the blood samples contain 100 mg sodium fluoride and 25 mg potassium oxalate as preservatives and the urine samples contain 1% sodium fluoride as a preservative. Before analysis, the samples are stored in a refrigerator.[8]

MATERIALS AND METHODS

Volunteers

There were totally 42 volunteers belonging to the age groups 22-44 years, 45-60 years and 61-76 years group. 3.2 Drug administration:

The volunteers were given an oral dose of zopiclone (5mg) tablet and blood collected at various intervals as follows: After administration, blood samples were collected at 0, 1, 2, 3, 4, 6, 12, 18, 24, 48h and plasma separated for zopiclone estimation.

After administration, after 2 hours the blood was collected and plasma separated,, which was stored in refrigerator for analysis at various intervals to see the stability of the zopiclone. Urinary levels of Zopiclone was also measured in all the groups.

Insomnia scale

After 24 hours of administration, the volunteers were asked about their sleep behaviour and how they feel about sleepiness or insomnia. They were given a score on the scale of 10, with 10 being more insomnia while 1 being less insomnia.

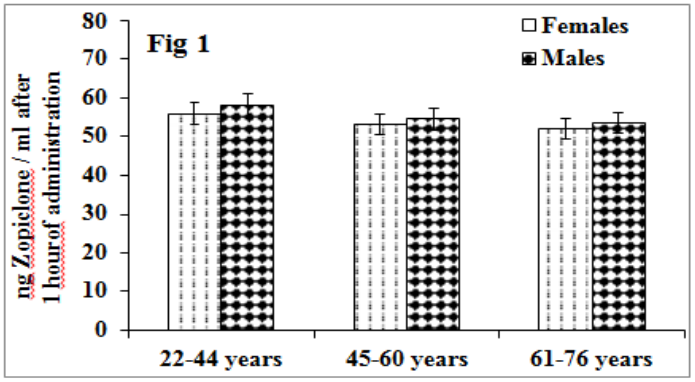
Spectrophotometric estimation of Zopiclone 100 µl of plasma was mixed well with 200 ul of butanol. Then this was centrifuged at 2500 rpm for 10min at 4C. The supernatant 100µl was taken and diluted to 1ml by adding 0.9ml of butanol. The optical density of this

extract was measured at 251 and 301 nm (reference). A standard curve of pure Zopiclone was used to calculate the sample readings.

Statistical analysis

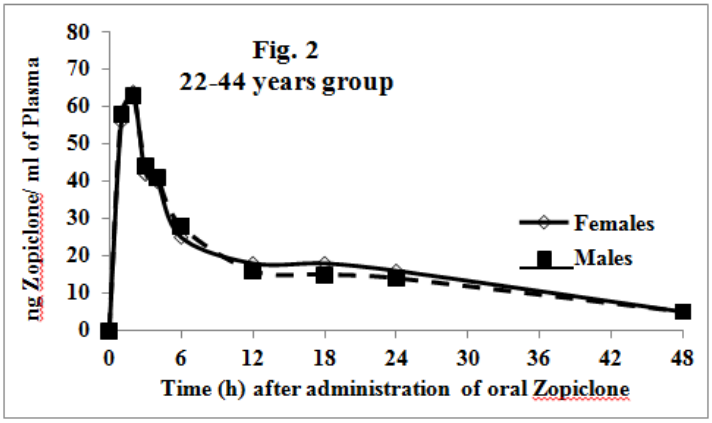
The data expressed as mean ± SE were analyzed by one-way ANOVA followed by a post hoc Tukey's test to compare the control and treatment groups as well as among the groups ($p \leq 0.05$) using GraphPad Prism 5.0 software. Different alphabet letters indicate significance difference among the respective groups. In some assays, * indicates significance difference from control ($p \leq 0.05$).

RESULTS



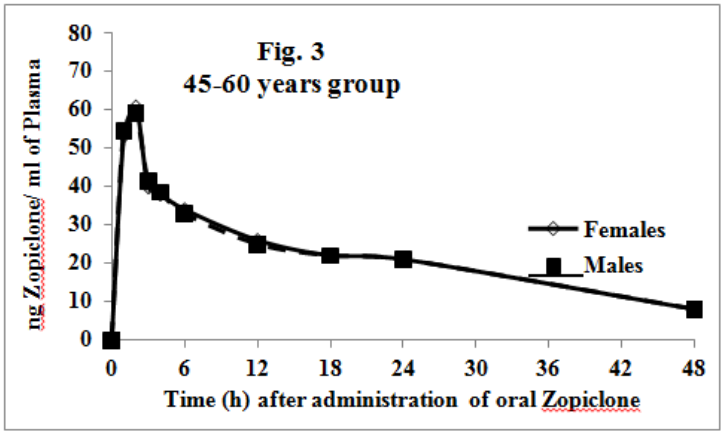
No much difference in Zopiclone concentration among Males and Females.

Fig. 1: Concentration of Zopiclone in the plasma after 1 hour of administration (single oral dose of 0.5mg).



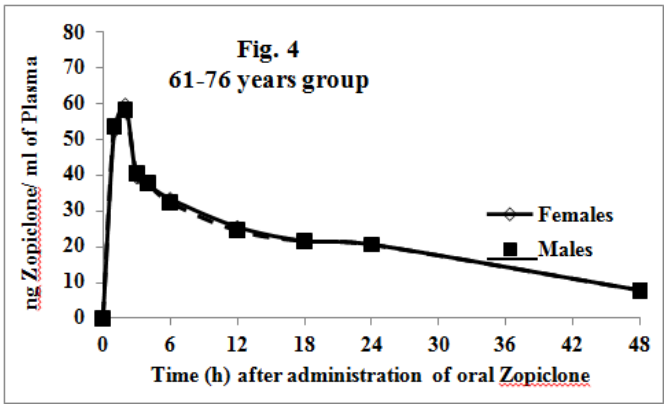
Zopiclone completely disappeared after two days of single injection (plasma).

Fig. 2: Pharmacokinetic assessment of Zopiclone in plasma from 0-48 hours after single administration.



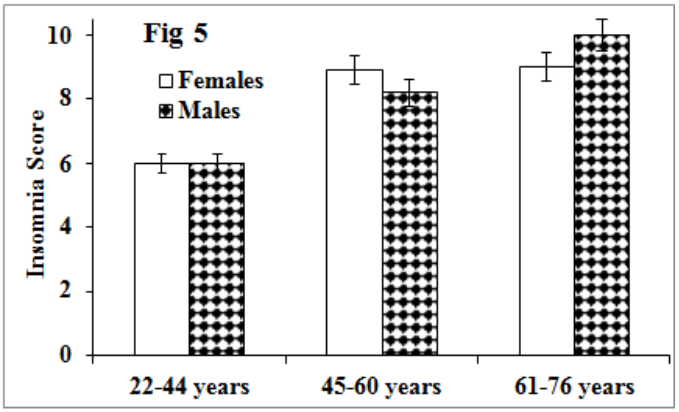
In the middle age group, the plasma levels decreased slowly, indicating the lower detoxification pathways (probable explanation).

Fig. 3: Pharmacokinetic assessment of Zopiclone in plasma from 0-48 hours after single administration (45-60 years age group.



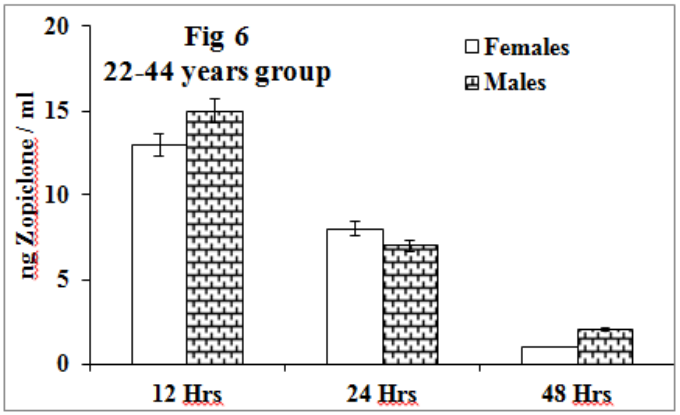
No much difference between elderly and middle aged group in processing the zopiclone.

Fig. 4: Pharmacokinetic assessment of Zopiclone in plasma from 0-48 hours after single administration (61-76 years age group).



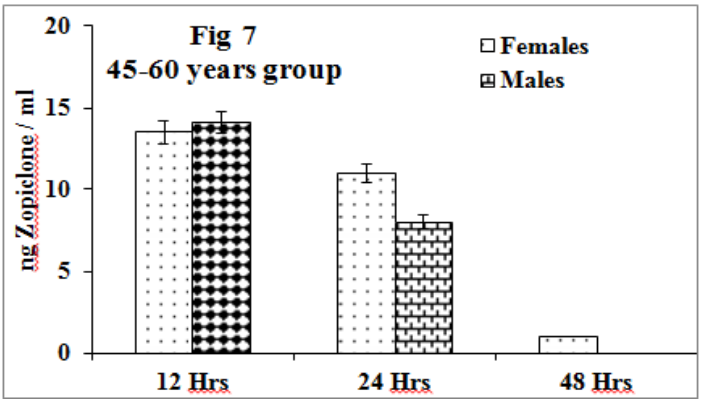
Middle aged and elderly people complained of sleeplessness (insomnia) the next day after zopiclone single injection. They wanted another dose for sleep inducing.

Fig. 5: Insomnia score among volunteers after 24 hours.



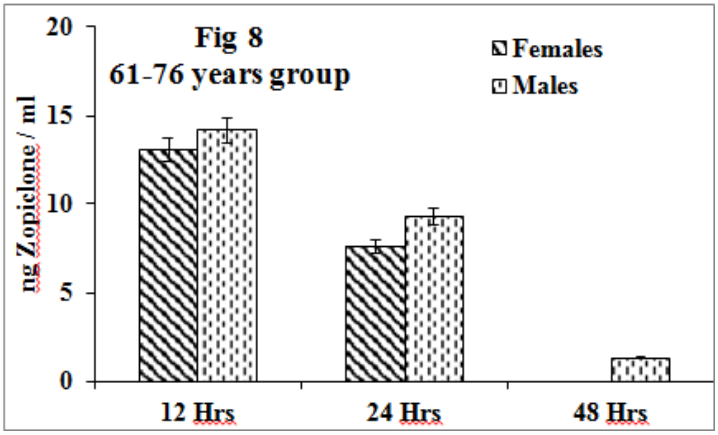
Urine analysis revealed the presence of traces after 48 hours also.

Fig. 6: Estimation of Zopiclone in urine samples of volunteers after oral administration (22-44 years group).



Complete absence urine of males in middle aged group.

Fig. 7: Assessment of Zopiclone excretion in urine samples of volunteers after oral administration (45-60 years group).



Complete absence among females in elderly. However, the fact is generally the urine levels diminish after 48 hours.

Fig. 8: Monitoring of Zopiclone urine levels from volunteers after oral administration (61-76 years group).

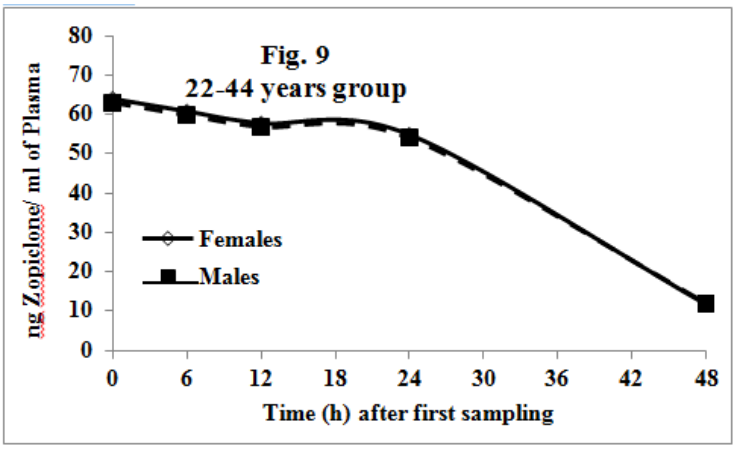


Fig. 9: Effect of storage on the Zopiclane stability among plasma samples (22-44 years group) sampled after 2 hours of administration, the plasma stored in refrigeration.

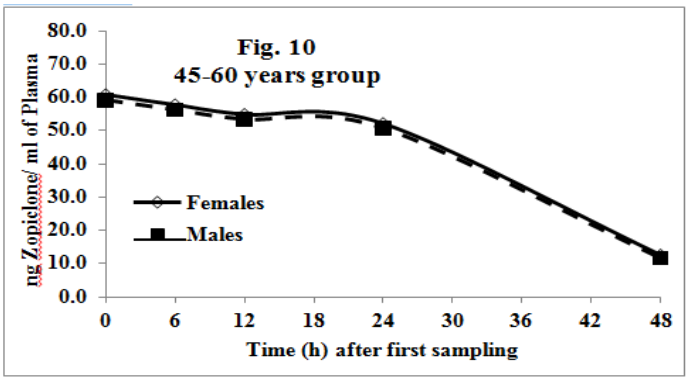


Fig. 10: Effect of storage on the Zopiclane stability among plasma samples (45-60 years group) sampled after 2 hours of administration, the plasma stored in refrigeration.

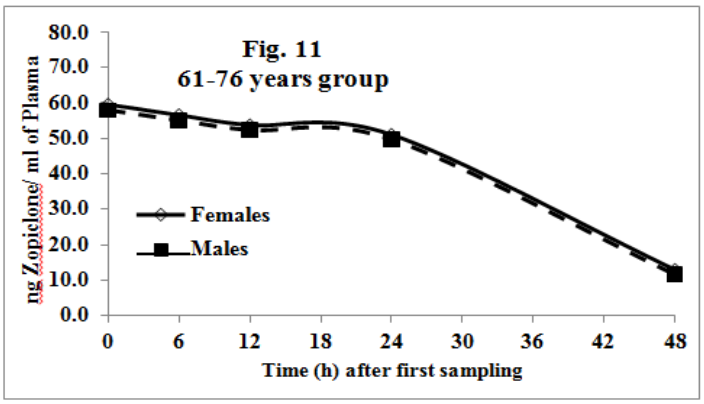


Fig. 11: Effect of storage on the Zopiclane stability among plasma samples (61-76 years group) sampled after 2 hours of administration, the plasma stored in refrigeration.

The levels of zopiclone got disappearing as the plasma was stored, in fridge. The same sample was analysed for zopiclone levels. This suggests that, zopiclone should be assessed as soon as the samples are collected.

DISCUSSION

In our study, we demonstrated that following the single administration of oral tablet of Zopiclone, the plasma levels diminish very slowly taking upto 48 hours. Some traces of Zopiclone were identifiable upto 72 hours (not presented here).

The major difference was the relapse of insomnia very fast among the elderly population and middle aged groups in comparison to the younger group of 22-44 years.

In addition, the storage of plasma even under refrigeration resulted in fast degradation of zopiclone in the samples. This suggests strongly that, the zopiclone should be estimated as the sample is fresh. In addition the zopiclone values from stored samples need verification with other assays.

Interestingly there was no much difference in the responses and the zopiclone stability between the samples from men and women in each age group participated.

However, zopiclone along with other drugs should be assessed as part of the forensic investigation.

CONCLUSION

Z-drugs have few distinct advantages over their predecessors, the benzodiazepines, and in many ways they have similar adverse and toxic effects, especially zopiclone. The effects of Z-drugs largely derive from their GABAergic action and pharmacokinetic profiles, which decide the extent of efficacy and toxicity. Adverse Z-drug effects and toxicity are more likely with polydrug use in therapeutics and co-ingested psychoactive substances in overdose. Z-drug poisoning is clinically similar to benzodiazepine overdose with supportive care sufficient in managing the majority of cases. The increasing ability to detect Z-drugs in various biological matrices is promising for future forensic endeavors. Postmortem redistribution appears to be significant for zolpidem and likely also for zaleplon. It is recommended that public health and drug regulatory authorities maintain a high level of toxicovigilance with regard to Z-drugs and their adverse outcomes.

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