
Hypno-sedative activity of *Gymnema sylvestre* extract in sodium barbiturate - induced hypnosis in *Rattus norvegicus*

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Abstract: *Gymnema sylvestre* is a rare herb with significant medical attributes and ethno-botanical uses. Insomnia is one of the most common sleep disorders around the world. In this study, the effect of the aqueous extracts of this plant was investigated for its hypno-sedative effect in rats as well as acute toxicity. Leaf, stem and flowers of *G.sylvestre* have several therapeutic applications in folk medicine in curing or managing of a wide range of diseases including insomnia. Sleep was induced using sodium barbiturate in albino rats. Rats of either sex weighing between 100-115g were grouped into five groups A,B,C,D and E of four animals each. Group A was the negative control and received only water, groups B the positive control received 10mg/kg b.w of diazepam .Groups C,D and E were administered 150,700 and 1500mg/kg b.w of extracts respectively, each of the rat was placed in individual cage and observed over a period of 24 hours at 6 hours interval .The aqueous extracts produced significant sedative effect at the dose of 150mg/kg b.w and was more effective than diazepam. The extracts at the doses of 150 and 700mg/kg b.w exhibited hypno-sedative activity comparable to the reference drug. The stem extract at 1500mg/kg produced no sedative effect. No toxicity signs were observed following acute toxicity evaluation. The extracts showed hypno-sedative activity at lower doses of 150mg/kg b.w in this increasing order, stem→leaf →flower. In conclusion, these results suggest that the aqueous extract of *G. sylvestre* is relatively non-toxic and possess potent sedative and hypnotic effects which could support its therapeutic use for insomnia in folk medicine.

Keywords: Hypno-Sedative, Sodium Barbiturate, *Gymnema sylvestre*, *Rattus norvegicus*

1. Introduction

Nigeria is most fortunate to have varied climate that almost any medicinal plant can grow. A medicinal plant is any plant which in which one or more of its organs, contain substance that can be used for therapeutic purpose or which are precursor for chemo-pharmaceutical semi-synthesis. Healing with medicinal plants is as old as mankind. The use of medicinal plants as fundamental component of the African traditional health care system is perhaps the oldest and most assorted of all the therapeutic systems.

Insomnia is defined as persistent difficulty in falling or staying asleep that affects function and can induce significant psychological and physical disorder. Sedatives are drugs that decrease activity and have a calming, relaxing effect. At higher dose, sedatives usually cause sleep. Drugs used mainly to cause sleep are called hypnotics. The difference between sedative and hypnotics then, is usually the amount of the dose; lower doses have a calming effect and higher

doses cause sleep (Huang et al., 2007)

Insomnia is a frequent problem that affects people of all the ages around the world. It is a prevalent and potentially serious condition that adversely affects the diurnal functioning, health status and life quality of people of all ages (Edinger and Means,2005). Stress, anxiety and depression could trigger insomnia. As well as cough, chronic pain, apnea, circadian rhythm disorders and neural diseases are conditions also associated with insomnia (Harvey,2001).

Insomnia is treated pharmacologically and non-pharmacologically or with a combination of both (Benca, 2005). Benzodiazepine, zolpidem, zopiclone, zaleplon have been used for the treatment of insomnia (Gottesmann, 2002). Relaxation, stimulus control, sleep restriction and sleep hygiene are common behavioral therapeutics for insomnia (Nan et al., 2005).

Medicinal plants constitute another option to treat insomnia. Around the world have been used several plants like sleep inducers such as valerian, passion flower, Melissa, Hops and Kava-Kava (Banned) (Wheatley, 2005).

Recent studies have shown that herbal drugs exert good sedative and hypnotic effect on central nervous system (Huang *et al.*, 2007; Herrera-Ruiz *et al.*, 2007; Perez-ortega *et al.*, 2008).

Gymnema sylvestre is a perennial wood climber which belong to the family *Asclepiadaceae* or the milk weed family (Kanetka *et al.*, 2007). *G. sylvestre* has several medicinal attributes. It is presently used traditionally as tea bags, beverages and confectionaries and it is also a good source of bioactive substances (Ch *et al.*, 2012).

Traditionally the leaves of *G. sylvestre* were used for the treatment of diabetes and other disorders, while the flowers and bark are given in disease related to phlegun (Kirtika and Basu, 1975). Reports in the ancient literatures suggested that the plant has multiple medicinal applications namely, antipyretic cardio tonic, liver tonic, digestive diuretic, cough dyspepsia laxative, stimulant etc the root bark is useful as an enemic, expectorant and analgesic for body ache and root juice in the treatment of snake bite (Anis *et al.*, 2000). The plant also exhibits medicinal importance in the treatment of constipation, cardiopathy asthma, bronchitis, conjunctivits and vesical calculi etc (Anis *et al.*, 2000).

The leaves of *G. sylvestre* contain triterpene saponins belonging to oleanane and damarane classes. The major constituent like gymnemic acids and gymnema saponins are members of oleanane type of saponins while gymnemasides are damarane saponins (Foster, 20002). There have been speculations in ethno- medicine that *Gymnema sylvestre* leaf extract cause sedation in diabetic patients. Sedation is the reduction of irritability or agitation by the means of administration of sedative drugs. Hypno-sedation reduces that need for general anesthesia (Brown *et al.*, 2010).

The aim of this study was to evaluate the sedative and hypnotic activities of *Gymnema sylvestre* aqueous extracts in sodium barbiturate induced hypnosis in *Rattus novergicus*, and to therefore, determine the scientific basis for its use in traditional medicine in the management of central nervous system disorders.

2. Materials and Methods

Plant material- The leaf, stem and flower of *Gymnema sylvestre* were collected based on ethno pharmacological information from the villagers in Ajokuta Local Government Area of Kogi State, Nigeria. The plant had already been authenticated by Dr, William D. Hawthorne, a James Martin Research fellow, Plants for the 21st century, Department of plant sciences, University of Oxford. The collected plant parts were rinsed with clean water to remove dirt. The leaves and flowers were plucked from the stem and were separately spread and air-dried under shade at room temperature for three (3) weeks.

2.1. Preparation of Plant Extracts

The air-dried leaves and flowers were pulverized into powder using an electric blender while the stem was first pounded into semi-powder with mortar and pestle, which

was further pulverized into fine powder utilizing mechanical grinder. Cold extraction method was used to obtain aqueous extracts of the samples. Portions (197.7g) of leaf, 271.15g of powdered stem and 103.89g of powdered flower were soaked in different containers with 2000ml of distilled water each. They were properly stirred and left for four (4) days with continuous stirring each day. The mixtures were then filtered using the high pressure vacuum pump machine. The filtrates in beakers were concentrated by evaporation at 60°C to dryness in a water bath.

2.2. Animals

The experimental animal used was albino rats (*Rattus novergicus*) of either sex weighing between 100-115g. They were obtained from a breeder at Kogi State University Staff Quarters, and housed in wired cages in the animal house of the Department of Biochemistry, Faculty of Natural Sciences Kogi State University, Anyigba, Nigeria and were acclimatized for two weeks prior to the commencement of the experiments. The animals were housed under standard laboratory conditions, light and dark cycles of 12h, and were provided with standard rodent pellet diet and water *ad libitum*. The animals were categorized into control and experimental groups. Group A was the negative control and received only water while group B was the positive control which received 10mg/kg of diazepam. Groups C,D, and E were administered 150, 700 and 1500mg/kg b.w of extracts respectively. There were four rats per group.

Appropriate authority has consented to the use of these animals in the Department of Biochemistry for experimental purpose.

2.3. Drugs

All drugs were freshly prepared on the day of experiments. Diazepam was obtain from Kuzak Pharmacy store in Anyigba, Kogi State, Nigeria and was of the Pfizer product. Sodium barbiturate was obtained from British Drug House. The other reagents were of analytical grade.

2.4. Acute Toxicity Study

The median lethal dose (LD₅₀) values were determined as described by Litchfield and Wilcoxon (1949). The acute toxicity test was carried out on the rats to evaluate any possible toxicity. Seven groups of rats numbering four rats per group of either sex were treated with different doses (150,700,1500 2000, 2500, 5000 and 6,000mg/kg p.o) . After a single dose administration the rats were placed in individual cages and were observed for 24hours and 6 hour time interval to detect any eventual side effects. The method estimated the dose of the extract that would kill 50% of a reduced sample of animal by a given route.

In the first phase, the extract was given to four rats per group at the doses of 150, 700, 1500, and 2000mg/kg; when no mortality was observed, the doses were increased to 2,500, 5000 and 6,000mg/kg. Rats were kept under further observation for 14 days to register further possible mortality.

2.5. Hypno-Sedative Activity Study

Rats of either sex were acclimatized for two weeks. The animals were divided into five groups of four rats each.

Groups A. Treated with distilled water (1ml/kg p.o) which served as control

Group B. Served as standard (treated with 10mg/kg b.w p.o diazepam)

Group C. Treated with 150mg/kg b.w p.o extract.

Group D. Treated with 700mg/kg b.w p.o extract.

Group E. Treated with extract at 1500mg/kg b.w p.o

After 60minutes of the previous treatments, 50mg/kg of sodium barbiturate was administered to all the animals to induce sleep. Each animal was observed for onset and duration of sleep. The duration of sleep or hypnosis was considered the loss of postural reflex. The effect was recorded for disappearance (on set of sleep) and reappearance (duration) of the righting reflex. Hypnotic sleeping time was considered to be the time interval between disappearance and reappearance of the righting reflex (Herrera-Ruiz et al.,2007).

3. Statistical Analysis

The statistical analysis was done using analysis of variance (ANOVA) and students t-test was carried out on data and ($p < 0.05$) was considered significant. The data are expressed as mean \pm S.D

4. Results /Discussion

4.1. Acute Toxicity of *G. sylvestre* Aqueous Extracts

Following oral administration of the aqueous extracts of *G. sylvestre* extracts at the doses of 150, 700, 1500, 2000, 2500, 5000 and 6000mg/kg P.O. There was no toxicity sign observed and no significant changes in the body weight between the control and treated group were observed at these doses. This result clearly indicates that the LD₅₀ was higher than 6000mg/kg. This is an indication of relative safety.

4.2. Hypno-Sedative Activity of the Extracts

Table 1. Effect of the aqueous leaf extract of *G. sylvestre* on the onset and duration of sleep in sodium barbiturate treated rats.

Group	Treatment/ dose (Mg/kg)	Sleep latency (minutes)	Sleep time (minutes)	Duration (minutes)
A	Water (1ml/kg)	34.50 \pm 0.58	66.00 \pm 1.16	31.50 \pm 0.58
B	Diazepam (10mg/kg)	20.00 \pm 0.00 ^a	72.50 \pm 2.86	52.00 \pm 2.89 ^a
C	150mg/kg extract	15.00 \pm 0.50 ^b	87.50 \pm 8.66	72.50 \pm 8.66 ^b
D	700mg/kg extract	13.50 \pm 1.00 ^c	72.25 \pm 4.50	58.75 \pm 3.50 ^c
E	1500mg/kg extract	3.75 \pm 0.50 ^d	59.75 \pm 7.50	56.00 \pm 7.00 ^d

Values with different superscript in a column are statistically significant ($p < 0.05$). Values are mean \pm S.D of four replications.

Table 2. Effect of the aqueous stem extract of *G. sylvestre* on the onset and duration of sleep in sodium barbiturate treated rats.

Group	Treatment/ dose (Mg/kg)	Sleep latency (minutes)	Sleep time (minutes)	Duration (minutes)
A	water (1mg/kg)	34.50 \pm 0.58	66.00 \pm 1.16	31.50 \pm 0.58
B	Diazepam (10mg/kg)	20.00 \pm 0.00 ^a	72.50 \pm 2.86	52.00 \pm 2.89 ^a
C	150mg/kg extract	49.50 \pm 1.73 ^b	14.50 \pm 4.00	35.50 \pm 2.27 ^b
D	700mg/kg extract	47.00 \pm 1.16 ^c	6.25 \pm 1.50	40.75 \pm 0.34 ^c
E	1500mg/kg extract	0.00 \pm 0.00 ^d	0.00 \pm 0.00	0.00 \pm 0.00 ^d

Values with different superscript in a column are statistically significant ($p > 0.05$). Values are mean \pm S. D of four replications.

Table 3. Effect of the aqueous flower extract of *G. Sylvestre* on the onset and duration of sleep in sodium barbiturate treated rats

Group	Treatment/ dose (Mg/kg)	Sleep latency (minutes)	Sleep time (minutes)	Duration (minutes)
A	Water (1m/lkg)	34.50 \pm 0.58	66.00 \pm 1.16	31.50 \pm 0.58
B	Diazepam (10mg/kg)	20.00 \pm 00 ^a	72.50 \pm 2.86	52.00 \pm 2.89 ^a
C	150mg/kg extract	14.75 \pm 0.50 ^b	217.50 \pm 2.70	202.75 \pm 4.81 ^c
D	700mg/kg extract	13.75 \pm 0.89 ^c	193.50 \pm 4.90	179.75 \pm 4.81 ^c
E	1500mg/kg extract	0.50 \pm 0.58 ^d	171.00 \pm 13.86	170.50 \pm 13.28 ^d

Values are mean \pm S. D, n=4. Values with different superscript in a column are statistically significant ($p < 0.05$).

Table 1, showed significant ($p < 0.05$) dose- dependent decrease in the latency of sleep time of animals which received the extract.

A difference of 19.50, 21.00 and 30.75 minutes in groups C, D and E from the control value of 34.50 \pm 0.58 minutes in group A. Animals in group B which received diazepam, a standard drug gave a difference of 14.50minutes in latency of sleep time from the control value. There was also dose-

dependent significant ($p < 0.05$) decrease in the duration of sleep time in animals that were administered the extract in groups C, D and E, the duration of sleep time were 72.50 \pm 8.66, 58.75 \pm 3.50 and 56.00 \pm 7.00 respectively. Animals which were administered diazepam had duration of sleep time of 52.00 \pm 2.89 while animals in group A had duration of sleep time of 31.50 \pm 0.58 respectively.

In table 2, there was a dose - dependent significant ($P <$

0.05) decrease in the latency of sleep time among animal in group C, D and E which received the stem extract at doses of 150, 700 and 1500mg/kg b.w. the latency of sleep time was 49.50 ± 1.73 , 47.00 ± 1.16 and 0.00 ± 0.00 minutes respectively. The treatment caused a non-significant ($p > 0.05$) effect in the duration of sleep time of 35.50 ± 2.27 minutes in group C and 40.75 ± 0.34 minutes in group D. In group E the animals were completely awake.

In table 3, the latency of sleep time decreased in a significant ($p < 0.05$) dose -dependent manner of animals in group C, D and E which were administered doses of 150, 700 and 1500mg/kg of the extract, the latency of sleep times were (14.75 ± 0.05 , 13.75 ± 0.89 , and 0.05 ± 0.58 minutes) respectively. This effect compares with animals in group B which received diazepam and had latency of sleep time of 20.00 ± 0.00 minutes. The treatment had significant ($p < 0.05$) dose - dependent decrease in the duration of sleep time in group C, D and E. The duration of sleep time were 202.75 ± 2.65 , 179.75 ± 4.81 and 170.5 ± 13.28 minutes respectively.

Up to a dose of 6000mg/kg b.w administered, the animal showed no signs of toxicity, indicating that the extracts used were relatively safe and caused no observable toxic effect. In this investigation, we observed the sedative and hypnotic properties of aqueous extract from *G. sylvestre* in rats. Diazepam is central nervous system depressant used in the management of sleep disorders such as insomnia; these compound have a binding site on GABA receptor type A-ionophore complex (GABA_A) (Huang *et al.*, 2007; Herrera-Ruiz *et al.*, 2007).

It decreases activity, moderates excitement, and calms the recipient. Substances like diazepam (which has been selected as the standard reference drug in this investigation) reduce latency or onset of and increase duration of barbiturate induced sleep and reduce activity possessing potentials as sedative (File and Wardill, 1975; Roy-Byrne, 2005).

The latency of sleep time significantly ($P < 0.05$) decreased with increase in dosage of the extracts, this may be attributed to an inhibition of the barbiturate metabolism or to an action in the regulation of sleep extracts at lower dose. (150mg/kg) caused hypno-sedation, it becomes imperative to try this experiment with doses lower than 150mg/kg. However, high dose of the leaf extract (1500mg/kg) exhibited non-significant ($p > 0.05$) sedative effect comparable to diazepam (table 1).

The anti-hypno-sedative effect of the stem extract at 1500mg/kg b.w is evident by the 24hours lost of sleep (insomnia) by animals in group E (Table 2). The latency of sleep time significantly ($P < 0.05$) decreased with increase in the doses of extract, however, the duration of sleep time were more prolonged in animals which received the flower extract (Table 3). All sedative effect peaks at 150mg/kg b.w. Observation from this study showed that *G. sylvestre* extracts could possess both hypno-sedative and anti-hypno-sedative properties. The order of increasing sedative activity of the plant parts is stem → Leaf → flower. The speculation of the ethno-medical practitioners on the sedative properties of *G.*

sylvestre extract might not be wild.

Further pharmacological analysis of the extracts will be conducted to isolate and characterize the active principles responsible for the sedative and hypnotic effect.

5. Conclusion

In conclusion, acute study showed that the plant parts are relatively non-toxic. Oral administration of aqueous extract of *G. sylvestre* induces sedative effects, supporting its use in folk medicine. Since the LD₅₀ values of the extract was above 6000mg/kg b.w for oral administration as determined following the method described by Litchfield and wilcoxon 1949; these results indicate a remote risk of acute toxicity and good tolerance of these extracts in traditional medicine.

In summary this present work represents that aqueous extract of *G. sylvestre* have obvious sedative and hypnotic activity and at higher doses can keep organism awake. The presented data provides pharmacological evidence for its therapeutic use on insomnia and could be added to the list of Nigeria medicinal plants for further screening.

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