SOME CENTRAL NERVOUS SYSTEM EFFECTS OF THE AQUEOUS LEAF EXTRACT OF *SOLANUM NIGRUM,* LINN (SOLANACEAE).

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ABSTRACT

Solanum nigrum Linn (Solanaceae) commonly known as deadly nightshade is a plant native to Eurasia and found in all the dry parts of India. It has a long tradition of use for its sedative and analgesic properties. However, no pharmacological basis has been provided for these ethnomedicinal applications hence this study.The leaves of the plant were extracted using distilled water and screened for sedative properties via barbiturate-induced sleeping time and motor coordination. The animals were divided into six groups of five animals per group and administered with the extract in graded doses (100, 200 and 400 mg/kg). Central analgesic effect was also investigated via the use of the hotplate analgesic meter. In this case, the duration of time spent by each animal on the hot plate before licking its paws was observed and recorded accordingly. Comparison was made with control groups that were administered with caffeine, diazepam, pentazocine and normal saline. The phytochemical constituent of the plant was also determined using various laboratory test methods and reagents. The oral acute toxicity profile of the extract was also determined, where the animals were closely observed for the first 24 hours and thereafter for the next fourteen days. The results obtained revealed that the aqueous leaf extract of *Solanum nigrum* significantly ($p<0.05$) prolonged both the duration of sleep in the barbiturate- induced sleeping time and time spent on the rotarod at the 400 mg/kg dose, while the 100 mg/kg dose showed significant ($p<0.05$) stimulant effect via reduction of the duration of sleep in the barbiturate induced sleeping time. All doses of the extract significantly ($p<0.05$) enlongated the mean reaction time spent on the hot plate from 120th min indicating protection against centrally induced pain. It also showed a wide therapeutic window, with a mean lethal dose of 2.3 g/kg.It can be concluded that the plant does possesses some central nervous system effects and is fairly safe.

Keywords: Stimulant effect, centrally pain, depressant effect, acute toxicity

INTRODUCTION

Herbal medicine also called botanical medicine or phytomedicine refers to the use of a plant's seeds, leaves, flowers, roots, bark or berries for medicinal purposes. The plants used are referred to as herbs. Simply put, an herb is a plant or plant part used for its scent, flavour or therapeutic properties (WHO, 2009).

Many herbs have been used for a long time for claimed health benefits. Herbalism has a long tradition of use outside of conventional medicine and it is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treatment and prevention of disease. An example of such a medicinal herb currently being used and undergoing research for its acclaimed therapeutic activity is *Solanum nigrum* commonly known as deadly nightshade, black nightshade, makoy or kakamachi. It belongs to the family Solanaceae, order Solanels, class Magnoliopsida, division Magnoliophyta, and kingdom

plantae.

Although *Solanum nigrum* is a rich source of one of plants most dreaded toxins solanine, it has appreciably demonstrated its potential as a reservoir of antioxidants having hepatoprotective, anti-tumor, cytostatic, anti-convulsant, anti-ulcerogenic and anti-inflammatory effects. Black nightshade (*Solanum nigrum*) is known to be used for its antipyretic, analgesic and most commonly its anti-inflammatory property (Cai *et al.*, 2010).

Recent studies on the aqueous leaf extract of the herb suggest its use in the management of seizures (Wannang *et al.,* 2008). An infusion of *solanum nigrum* is used as an enema in children suffering from abdominal upset.

The chloroform extract of *Solanum nigrum* is also known to be used for its antinociceptive, anti-inflammatory and antipyretic effects (Zakaria *et al.*, 2006).

Solanine poisoning causes dizziness hence the plant is being investigated for its folkloric use as a sedative (Blomqvist and Nguyen, 1999).

The purpose of this study is to investigate the use of the aqueous leaf extract of *Solanum nigrum* as a sedative and central analgesic agent in order to provide a pharmacological basis for its ethno medicinal applications.

MATERIALS AND METHODS
Collection of plant and identification
The fresh leaves of *solanum nigrum* herb was collected from Okhoro village, Egor local government area of Edo state, Nigeria and identified by Mr. Sunny Nweke a herbarium curator in Pharmacognosy department, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria and a voucher specimen deposited. Immediately after collection, the leaves were air dried and pulverized into a smooth powder using an impact mill and kept for further analysis.

Drugs and chemicals
Thiopentone (Rotexmedica Trittau Germany), caffeine (BDH Chemicals), diazepam (Swipha, Roche), Pentazocine (Embassy), normal saline, distilled water, stock solutions of the various drugs and the aqueous leaf extract of *Solanum nigrum* were prepared fresh for each experiment.

Animals
Swiss mice (*Mus musculus*) weighing 22-35g and adult Wistar rats (*Rattus novegicus*) weighing 150-280g of both sexes kept at the laboratory animal house of the department of anatomy, University of Benin were used. The animals maintained under standard environmental conditions had access to standard diet (Top feed, Premiere feed mills company limited) and water *ad libitum.* Animals were kept in a cage with a twelve hour light-dark cycle. All experiments were performed after an overnight fast and conformed to acceptable protocols for use of animals in experiment.

Ethical approval
Approval for the use of the animals was obtained from the ethical committee on the use of animals, Faculty of Pharmacy, University of Benin, Benin City, Edo State. Nigeria.

Extraction of plant material
The powdered plant material (300 g) was extracted with distilled water via hot decoction and then filtered. The filtrate was concentrated to dryness over a hot water bath for 8 hours. The concentrated extract was stored in a refrigerator at 4^0C prior to use. Percentage yield obtained was 26.4 %.

Barbiturate-induced sleeping time
The animals were divided into six groups of five rats per group. The first four groups received intraperitoneally 0.2ml normal saline, 100, 200 and 400 mg/kg doses of the aqueous leaf extract respectively. The last two groups received subcutaneously 20 mg/kg caffeine and 0.1 mg/kg diazepam respectively. All treatments were done 30 minutes prior to an intraperitoneal administration of 40 mg/kg thiopentone. The onset of sleep and the duration of sleep for each animal were determined and mean for each group calculated. The onset of sleep is the interval between administration of thiopentone and loss of righting reflex while the duration of sleep is the time interval between onset of loss of righting reflex and regain of righting reflex.

Motor coordination

The effect of the extract on motor coordination was determined via the use of the Ugo Basile Rota rod bar. Swiss albino mice were divided into 6 groups of 5 mice per group after an initial screening. This screening involved placing each mouse on the Rota rod prior to treatment, any mice that fell before the cut off time of 2 minutes was excluded from the experiment. The aqueous leaf extract of *Solanum nigrum* (100, 200 and 400 mg/kg) were administered intraperitoneally to the first three groups. The fourth group received 0.2ml each of normal saline while the last two groups received 0.1 mg/kg diazepam and 20 mg/kg caffeine respectively. The animals were placed on the Rota rod bar prior to treatment and at 0.5, 1, 2, 3 and 4 h after treatment.

Central analgesic effect
The effect of the extract on central analgesic activity was determined via the use of the hot plate analgesic meter (Turner, 1965). Swiss albino mice were divided into 5 groups of 5 mice per group after an initial screening. This screening involved placing each mouse on the hot plate prior to treatment, any mice that licked its paw or jumped off before the cut off time of two mins was excluded from the experiment. 0.2ml of distilled water was administered intraperitoneally to each mouse in group A. Groups B, C and D were administered 100, 200 and 400 mg/kg doses of the aqueous leaf extract of *Solanum nigrum* respectively while group E was administered pentazocine at a dose of 30mg/kg. The animals were placed on the hot plate analgesic meter prior to treatment and at 30, 60, 90, 120,150 and 180 minutes after treatment. The duration of time spent on the hot plate analgesic meter before licking the paws was recorded.

Oral acute toxicity study
The toxicity of the aqueous leaf extract of *Solanum nigrum* on rats was determined by administration of graded doses of the extract to the animals (Lorke, 1983). Adult wistar rats were divided into 6 groups of 5 rats per group. Each rat in group A received 2ml of distilled water while animals in groups B, C, D, E and F received 800, 1600, 3200, 6400 and 12800 mg/kg doses of the aqueous leaf extract of *Solanum nigrum* respectively. The animals were closely watched for the first 24 hours and thereafter for the next fourteen days. Administration was done orally via an oro-gastric syringe.

Phytochemistry
The aqueous leaf extract of *Solanum nigrum* was screened for its phytochemical constituents (Trease and Evans, 1989).

Statistical analysis
Results were analyzed using the student t-test and was expressed as mean ± standard error of mean (SEM). Result was considered significant when $p < 0.05$ and very significant when $p < 0.0001$.

RESULTS
Phytochemistry
Table 1 shows the result obtained from the phytochemical screening of the aqueous leaf extract of *Solanum nigrum*. It revealed the presence of alkaloids and saponins, and the absence of tropane alkaloids, carbohydrate, phenolics and anthracenes.

Table1: Phytochemical screening of the

Test	Result
Carbohydrate	Absent
Alkaloids	Present
Tropane alkaloids	Absent
Phenolics	Absent
Saponins	Present
Anthracenes	Absent

aqueous leaf extract of *Solanum nigrum*
Barbiturate-induced sleeping time
Table 2 presents the mean and the standard error of mean values of the onset and duration of sleep in minutes induced by the different doses of the extract and the various other drugs administered. From the table, it could be seen that there was a significant reduction ($p < 0.05$) in the onset of sleep by the different doses of the extract. The 100 mg/kg dose produced a similar onset of sleep time in comparison with caffeine, however the 200 and 400

mg/kg dose had significantly lower (p<0.05) onset of sleep values in comparison with caffeine and diazepam treated groups. A significant (p<0.05) reduction in the duration of sleep with the 100mg/kg dose was observed in comparison with the normal saline groups. This effect was not significantly different from the caffeine treated groups, pointing to a similarity between the extract (100 mg/kg) and caffeine's effect. This is in contrast to what was observed with the 400mg/kg dose, here there was a significant (p<0.05) prolongation of the duration of sleeping time in comparison with the controls (normal saline) and caffeine treated groups. This effect of the extract at 400 mg/kg was similar to what was observed for the diazepam treated groups. However though the 200 mg/kg dose showed an increase in the duration of sleep, this was not significant.

Table 2: Effect of the aqueous leaf extract of *Solanum nigrum* on barbiturate-induced

Treatment (mg/kg)	Onset of sleep (mins)	Duration of sleep (mins)
Normal saline	15.2±7.54	86.40±17.51
SN (100)	8.13± 2.69[a]	55.75±20.18[a]
SN (200)	6.6±0.6782[b,ab,ac]	80.60±2.82
SN (400)	4.5±0.29[b,ab,ac]	179.25±34.25[b,ab]
Diazepam (0.1)	12.2±0.37	263.4±21.80[b]
Caffeine (20)	9.2±0.49[a]	66.25±17.38[a]

sleeping time
Values are mean ±SEM when n = 5.
[a]p<0.05 and [b]p<0.0001 significantly different from the normal saline group, [ab]p<0.05 significantly different than the caffeine treated group and [ac]p<0.05 significantly different than the diazepam treated group.

SN: *Solanum nigrum*

Motor coordination
Table 3 shows the effect of the aqueous leaf extract of *Solanum nigrum* on motor coordination. At the 100 mg/kg dose, it was observed that the mice spent significantly lesser time on the rotarod bar in comparison with the normal saline group. This effect was not significantly different from that of the caffeine treated group. At higher doses of the extract (200 and 400 mg/kg) the animals showed a gradual in-

crease in the time spent on the rotarod bar which however declined at the 4th hour. This effect in comparison with the control (normal saline was significant (p<0.05) between the 1st and 3rd hour. Those administered with diazepam and caffeine also showed similar patterns but with higher values in the group administered with diazepam. The effect of the extract at 400 mg/kg was more significant (P<0.05) than diazepam at the 1st and 3rd hour, with the animals spending more time on the rotarod bar.
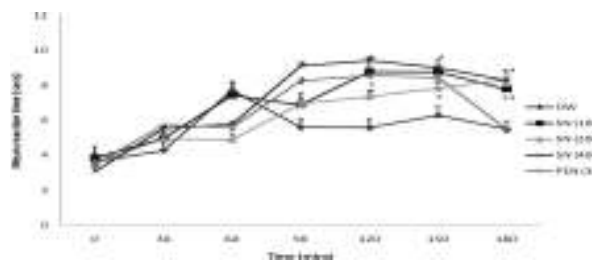
Table 3: Effect of the aqueous leaf extract of *Solanum nigrum* on motor coordination.

Treatment (mg/kg)	Time spent o the rotarod bar in secs at :				
	30mins	1hour	2 hours	3hours	4 hours
Normal saline	276.4±53.60	300±0.00	300±0.00	229.2±50.16	300±0.00
SN (100)	124.4±21.70[a]	153.6±76.42[a]	242±84.06[a]	159.4±74.49[a]	142.4±55.97[a]
SN (200)	115.8±62.63[a]	250.6±49.40[a]	343±0.00[b]	375.6±0.40[b]	330±0.00[b]
SN (400)	300±0.00	370±0.00[b]	260.2±58.27	320±0.00[b]	308±0.00
Diazepam(0.1)	191.4±69.03[a]	200.8±62.90[a]	266.6±51.66	220.4±59.01	300±0.00
Caffeine (20)	152.2±53.69[a]	216.6±63.07[a]	252.2±42.19	233.4±57.72	185±15.59[a]

Values are mean ±SEM when n = 5.
[a]p<0.05 significantly lower and [b]p<0.05 significantly higher than the normal saline group.
SN: Solanum nigrum

Hot plate reaction time
Figure 1 shows the effect of the extract at different doses on mean reaction time in seconds of mice. From the result, it could be seen that the effect of the extract at 400 mg/kg was most significant (p<0.05) from the 90th min. At 120th min, both the 200 and 400 mg/kg doses were significantly higher (p<0.05) than the control. However at 180th min, all the doses of the extract (100, 200 and 400 mg/kg) were significantly higher than the control (p<0.05,

p<0.0001, p<0.0001) respectively.
Fig. 1: Central analgesic effect of the aqueous leaf extract of *Solanum nigrum*
Values are mean ±SEM when n = 5.

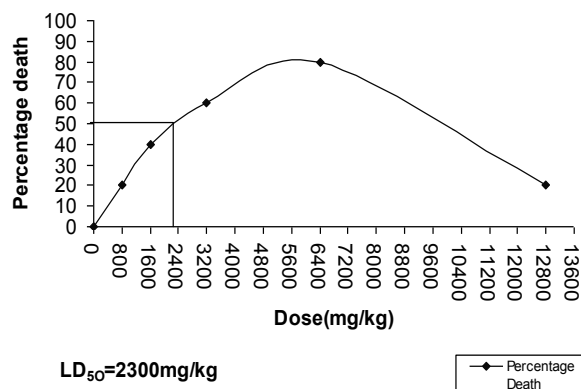[*]p<0.05 and [**]p<0.0001 significantly higher than the normal saline group.
DW: Distilled water
SN: *Solanum nigrum*
PEN: Pentazocine

Oral acute toxicity
Figure 2 presents the results of acute toxicity test done on rats.The LD_{50} of the extract was

LD_{50}=2300mg/kg

calculated as 2300mg/kg.
Fig. 2: Acute toxicity of the aqueous leaf extract of *Solanum nigrum*
Values are represented as n=5

DISCUSSION
Phytochemical analysis of the aqueous leaf extract of solanum nigrum
The phytochemical analysis of the aqueous leaf extract of *Solanum nigrum,* revealed that the extract contains saponins and alkaloids which are probably responsible for its pharmacological properties. The presence of alkaloids provides a basis for its central analgesic activity whereas the presence of saponins provides a basis for its antimicrobial properties (Muto *et al.,* 2006). The presence of alkaloids and saponins in the extract are suspected to be contributory to its pharmacological activity as an analgesic agent.

Barbiturate induced sleeping time of the aqueous leaf extract of solanum nigrum
Induction of sleep was by thiopentone a well known barbiturate that produces all degrees of depression of the Central nervous system, ranging from mild sedation to general anesthesia. Thiopentone in particular potentiates GABA-induced increases in chloride conduct-

ance and depresses voltage-activated Ca^{2+} currents (Beckstead *et al.*, 2000).

The animals administered with the 100 mg/kg dose of the aqueous leaf extract of *Solanum nigrum* had a longer onset and a shorter duration of sleep when compared with those administered with normal saline and diazepam. However, the 200 and 400 mg/kg doses of the aqueous leaf extract of *Solanum nigrum* produced a remarkable reduction in the onset of sleep and prolongation in the duration of sleep with the 400 mg/kg dose having a shorter onset and longer duration when also compared with the values obtained for the controls. This suggests that the aqueous leaf extract of *Solanum nigrum* has a dose dependent sedative effect i.e. the level of sedation increases with an increase in dose. However, at a very low dose, it tends to exert stimulant rather than sedative properties. This could be seen from the very high onset of sleep and short duration of sleep observed for the 100 mg/kg dose of the aqueous leaf extract of *Solanum nigrum* when compared with the values obtained for those administered with diazepam. The stimulant property of the 100 mg/kg dose of the aqueous leaf extract of *Solanum nigrum* was greater than that of caffeine while the sedative property of the 400 mg/kg dose of the aqueous leaf extract of *Solanum nigrum* was similar to that of diazepam. Caffeine being a known central nervous system stimulant was able to prolong the onset of sleep and reduce the duration of sleep when compared with those administered with diazepam and normal saline. Caffeine, a mild stimulant is the most widely used psychoactive drug in the world (Silverman *et al.,* 1999). It increases nor-epinephrine secretion and enhances neural activity in numerous brain areas. Many of its effects are believed to occur by means of competitive antagonism at adenosine receptors. Tolerance occurs rapidly to the stimulating effects of caffeine, thus a mild withdrawal syndrome has been produced. Herein lies the advantage of herbal remedies which most times are devoid of these withdrawal syndromes (Silverman *et al.,* 1999). Diazepam which is an anxiolytic and sedative agent reduced the time for sleep onset while also prolonging the duration of sleep in comparison with those in the negative control

group that were administered with normal saline.

Effect of the aqueous leaf extract of Solanum nigrum on motor coordination

A gradual increase in the duration of time spent on the bar with increase in the time of administration of the drug/extract was noted for all doses. This explains that while in the unmetabolized form, the activity of the drug was minimal. Activity gradually increased as the drug/extract was metabolized and also decreased as the drug was degraded by metabolizing enzymes. The CNS stimulant effect of the extract doses was further confirmed by its ability to maintain the animals on the Rota rod, thus indicating muscle co-ordination (Owolabi *et al*., 2007).

Central analgesic effect of the aqueous leaf extract of *Solanum nigrum*

The result obtained from the graph of mean reaction time in seconds against time in minutes in the test for the central analgesic effect of the aqueous leaf extract of *Solanum nigrum* using the hot plate analgesic meter*,* shows that the extract administered had no significance at 0 time, 30 minutes and 60 minutes. However from the 90th to 180th min, the extract significantly prolonged the reaction time at all doses tested. This indicates that the aqueous leaf extract of *Solanum nigrum* has a dose related effect on the control of centrally mediated pain. It is known that centrally acting analgesic drugs elevate the pain threshold of mice towards heat and pressure (Adeyemi *et al.,* 2004). From the above findings, the extract raised the pain threshold on the hot plate which indicates that it might be centrally acting. The extract thus seems to possess analgesic properties, which are mediated via central inhibitory mechanisms.

Acute toxicity of the aqueous leaf extract of Solanum nigrum

The result obtained from the acute toxicity study of the aqueous leaf extract of *Solanum nigrum*, shows that the highest number of deaths (80%) was recorded at a dose of 6400 mg/kg of the aqueous leaf extract of *Solanum nigrum* administered whereas the minimum lethal dose which is the dose that was observed to kill any experimental animal was

800 mg/kg. However, the LD_{50} which is also known as the median lethal dose reveals that 50% of the rats died when administered the aqueous leaf extract of *Solanum nigrum* at a dose of 2300 mg/kg. This suggests that aqueous leaf extract of *Solanum nigrum* has a wide therapeutic index and can thus be consumed without harm. The median lethal dose could also be used to calculate the initial dose to be tried in humans (Aaronson and Horvath, 2002).

The results obtained from this study permit the following conclusion: That the leaf extract possesses stimulant, sedative and central analgesic activities and thus could be employed as an alternative treatment to orthodox medicine.

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