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ORIGINAL ARTICLE

Development of *Nardostachys jatamansi* and *Withania somnifera* Formulation for Treatment of Sleep Disorders

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KEYWORDS

Sleep disorders;
Withaferin A;
Anxiety;
Insomnia;
Depression;
Catalepsy

ABSTRACT: Sleep is a state of decreased awareness of environmental stimuli, distinguished from states such as coma or hibernation by its relatively rapid reversibility. Insomnia and other sleeping disorders are quite common in the worldwide population nowadays. Although treatment with sedatives and hypnotics is available, the safety of those medications is a big question. In this present study, 2 different formulations developed by *Nardostachys jatamansi* and *Withania somnifera* at 2 different proportions are made and evaluated for the treatment of several sleep disorders. Isolation of marker compounds, evaluation of Pharmacokinetic parameters, and evaluation of the safety and efficacy of those 2 test formulations were performed. The results inferred that both formulations have potential therapeutic properties for prophylaxis and treatment of sleep disorders, as both formulations produced more than 50% sleep. Anxiety and cortisone levels were inhibited for more than 30% along with no signs of toxicity for oral administration at a 2g kg^{-1} dose, and other adverse effects associated with the use of traditional sedatives-hypnotics. Moreover, a reduction in total neurotransmitter levels in the brain, elevated due to sleep deprivation was also observed when maintaining levels below 50%. From these results, it was concluded that both formulations have a potential activity against sleep-related disorders without potential toxicological effects.

INTRODUCTION

Sleep is a state of decreased awareness of environmental stimuli that is distinguished from states such as coma or hibernation by its relatively rapid reversibility. Normal human sleep comprises two states - rapid eye movement (REM) and non-REM (NREM) sleep – that alternate cyclically across a sleep episode. About 35 percent of the population has difficulty falling asleep, maintaining sleep, early morning awakening, or non-restorative sleep, and in 10 percent of these insomniacs have a persistent problem interfering with daytime function. It is believed that during normal sleep, the metabolic rate reduces by around 15% and reaches a minimum in the morning in a standard circadian pattern [1]. Several symptoms are associated with sleep deprivation including changes in hormonal secretion profile (growth hormone, cortisol, insulin) may have profound effect on glucose regulation, sympathetic stimulation which might contribute to the metabolic dysregulation and inflammation due to

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alteration in immune response and increase pro-inflammatory markers such as Interleukin-6 (IL- 6), Tumor Necrosis Factor- α (TNF-α) and C-Reactive Protein (CRP) [2 – 4].

Although a large number of hypnotics were in clinical use at one time, most of them have become obsolete due to their associated serious adverse effects, including tolerance, dependence, hangover, ataxia, etc [5]. Currently, the available treatment options for management of sleep disorders are benzodiazepine and non- benzodiazepine based pharmacological agents. The traditional system of medicine utilizes many herbs to treat psycho-neurological disorders, including sleep disorders. Scientifically, sleep-promoting actions were reported with several medicinal plants, including *Bacopa monnieri*, *Centella asiatica*, *Hypericum perforatum*, *Ginkgo biloba*, *Withania somnifera*, *Nardostachys jatamansi*, *Rauvolfia serpentina*, *Valeriana wallichii,* etc. A previous review by Sharma et al. (2024) has signified the importance of *Nardostachys jatamansi* in treating central nervous system-related disorders, like convulsion, Parkinson's, depression, and insomnia. But no proper mechanistic insights have been identified for the exhibition of such pharmacological properties [6]. Among all those plants, two such herbs, *Nardostachys jatamansi* and *Withania somnifera*, which showed promising anti-stress, anxiolytic, anti-depressive, neuroprotective, and hypnotic action [7 – 11], were tried in combination under this research investigation.

MATERIALS AND METHODS

Animals required for the experiment

Adult Swiss albino mice and Wistar rats of both sexes were used and kept in CPCSEA-registered institutional animal house (R/N959/c/06/CPSEA) and maintained in accordance with CPCSEA guidelines [12]. The institutional animal ethics committee approved the protocol (RKC/IAEC/14/14, dated 7.9.2017). Only healthy animals were selected for the study.

Test drugs used

Two test drugs, SDA-217A and SDA-217B, were formulated and supplied by M/s Emami, Kolkata, to the PI, Department of Pharmacology, R.G Kar Medical College, Kolkata, for pharmacological studies on hypnotic/sedative action in animals. The details of the 2 used formulations are depicted in Table 1.

Table 1. Composition of the two different *Nardostachys jatamansi* and *Withania somnifera* formulations

Name of the formulation: SDA-217A (1:1) - code A	Name of the formulation: SDA-217B (7:3) - code B
Physical State: Light brown powder	Physical State: Light brown powder
Solubility: Sparingly soluble in water	Solubility: Sparingly soluble in water
Extract Ratio: *Withania somnifera* (1): *Nardostachys jatamansi* (1)	Extract Ratio: *Withania somnifera* (7): *Nardostachys jatamansi* (3)

HPTLC fingerprints of test drugs

Test drugs (SDA-217A and SDA-217B) were refluxed in methanol, reconstituted, filtered, and adjusted to 1 mg /ml. The samples were spotted in the form of bands on precoated silica gel plates (Merck, $60F_{254}$, 20 x 10 cm) using a CAMAG Linomat 5 applicator. Quercetin was used as a standard biomarker. The plates for BV were developed in a solvent system of toluene: ethyl acetate: formic acid=4.5:3:0.2 for 30 min. The densitometric scanning was performed on CAMAG TLC Scanner 3 at absorbance 254 nm by multi-level WinCATS planar chromatography manager [13, 14]. The area percentage of each separated compound with respect to R_f was plotted.

Test drugs preparation and route of administration

The test drugs were weighed accurately in glass conical flasks and mixed in an adequate amount of deionized water. The mixtures were shaken well using a vortex shaker for 30 min and filtered. The filtrate solutions were used for all pharmacokinetic/pharmacodynamic studies. The test samples were freshly reconstituted in the desired vehicles every day. The test samples were given orally using an epigastric feeding cannula for mice (2ml kg^{-1}) and rats (5ml kg^{-1}).

Pharmacokinetic study using HPLC

500 mg kg^{-1} p.o., a single large dose of the test drugs was administered to overnight fasted rats. 0.5 ml of blood

was withdrawn at 0, 5, 10, 30, 60, 120 min by occipital plexus, collected in Ethylenediaminetetraacetic acid (EDTA) tubes, and centrifuged at 5000 rpm for 10-15 min, post drug administration. Plasma samples were stored at -20ºC until the time of analysis by a High-Performance Liquid Chromatography (HPLC) system (Agilent Technologies, USA). Withaferine A (Sigma-Aldrich, USA CAS No.: 6119-48-2) was selected as a biomarker. The marker compound present in the test drug and rat plasma was quantified and compared [15 – 17].

Acute toxicity study

The formulations SDA-217A and SDA-217B were tested for an acute oral toxicity study following the guidelines of OECD No. 423 [18]. The test formulations were given to the 18 h fasted mice in doses of 0.5 g, 1.0 g, and 2.0 g per kg, i.e., in an arithmetically progressive manner by oral route in a single dose, and administered the animals were observed for 3 days. The test drug volume was restricted to 2 ml kg^{-1} per oral dose for each mouse. The behavioural and clinical signs & symptoms of toxicity were carefully observed and recorded during this period. Some of the major actions that were studied from this toxicity study include somatomotor activity, ataxia, tremor, convulsions, writhing, Straub, catalepsy, inclined plane test, alterations to pupils, ptosis, cyanosis, salivation, lacrymation, analgesia, diarrhoea, stereotypy and reflexes (pineal, corneal, and righting). The mortality (if any) was recorded up to 14 days for the determination of the 50% lethal dose of test formulations.

Hypnotic Study

Hypnotics, sedatives, and tranquilizers are known to prolong thiopental-induced sleep after a single dose. The loss of righting reflex is measured as a criterion for the duration of drug-induced sleeping time. Groups of pre-screened healthy rats with an average weight of 150-175 g were used. These were treated orally with the test drugs the reference standards, or the vehicle. The following doses were selected on the basis of previous pilot studies in our laboratory. The test drugs were given 5 days before experimentation. Experimental groups (N=6) were designed as mentioned in Table 2.

After 1 h of administration of the last dose, all animals were induced hypnosis with i.p. injection of thiopental 35 mg kg^{-1} body weight. The rats were placed on their backs on a warmed (37°C) pad, and the duration of loss of the righting reflex (starting at the time of injection) was measured until they regained their righting reflexes [19, 20]. The time to lose of the righting reflex and the gap between the loss and regain of the righting reflex were noted in seconds. The percent change in sleep pattern was calculated.

Table 2. Experimental grouping of animals for conducting the Hypnotic study.

Group	Test drug	N	Dose	Route	Pretreatment
Group 1	Normal Control-Distilled water	6	5 ml kg^{-1}	oral	5 days
Group 2	Positive Control - Diazepam	6	4 mg kg^{-1}	oral	5 days
Group 3	SDA-217A	6	125 mg kg^{-1}	oral	5 days
Group 4	SDA-217A	6	250 mg kg^{-1}	oral	5 days
Group 5	SDA-217A	6	500 mg kg^{-1}	oral	5 days
Group 6	SDA-217B	6	125 mg kg^{-1}	oral	5 days
Group 7	SDA-217B	6	250 mg kg^{-1}	oral	5 days
Group 8	SDA-217B	6	500 mg kg^{-1}	oral	5 days

Anti-depressant study

Adult Swiss mice (25 g) were forced to swim inside the bath-tub of 24-inch diameter and 12-inch height, containing water at 24-26°C for 2 min. Thereafter, during the next 4 min, the total period of immobility, characterized by complete cessation of swimming with the head floating above water level, was recorded. The group and division of animals for the experiment are elaborated in Table 3.

The depression score (immobility time 2-6 min) was calculated [21, 22]. After completion of the study, all animals were sacrificed by dislocation. The blood was collected, and plasma was separated for estimation of corticosterone by an ELISA kit for rats (Shanghai Coon Koon Biotech Limited, Shanghai, China). Later on, after brain isolation, lipid peroxidation, SOD, GSH, and protein in the brain were determined spectrophotometrically, [23] and estimation of neurotransmitters was performed by ELISA kits.

Table 3. Experimental grouping of animals for conducting an Anti-depressant study.

Group	Test Drug	N	Dose	Route	Pretreatment
Group 1	Normal Control-Distilled water	6	2 ml kg^{-1}	oral	5 days
Group 2	Positive Control - Imipramine	6	15 mg kg^{-1}	oral	5 days
Group 3	SDA-217A	6	125 mg kg^{-1}	oral	5 days
Group 4	SDA-217A	6	250 mg kg^{-1}	oral	5 days
Group 5	SDA-217A	6	500 mg kg^{-1}	oral	5 days
Group 6	SDA-217B	6	125 mg kg^{-1}	oral	5 days
Group 7	SDA-217B	6	250 mg kg^{-1}	oral	5 days
Group 8	SDA-217B	6	500 mg kg^{-1}	oral	5 days

Hyperactivity or Anxiety Study

The rats were subjected to foot-shock (2 mA, 10 shocks/day within 1h) for 14 days through a grid floor in a standard conditioning chamber with the escape route closed. The group and division of animals for the experiment are given in Table 4.

The animals were placed on the Plus Maze apparatus for anxiety score after the last test drug dose. The maze has two opposite open arms (50×10 cm), crossed with two opposite enclosed arms of the same dimension but having 55 cm high walls. The arms are connected with a central square, 10×10 cm, giving the apparatus the shape of a plus sign. The whole maze is elevated to a height of 50 cm. After 5 min of observation, anxiety scores were given to animals [24, 25], and later on sacrificed for spectrophotometrical analysis of lipid peroxidation, SOD, GSH, and protein in the brain [23] as well as neurotransmitter estimation.

Table 4. Experimental grouping of animals for conducting a hyperactivity or anxiety study.

Group	Test Drug	N	Dose	Route	Pretreatment
Group 1	Normal Control-Distilled water	6	5 ml kg^{-1}	oral	14 days
Group 2	Positive Control - Diazepam	6	4 mg kg^{-1}	oral	14 days
Group 3	SDA-217A	6	125 mg kg^{-1}	oral	14 days
Group 4	SDA-217A	6	250 mg kg^{-1}	oral	14 days
Group 5	SDA-217A	6	500 mg kg^{-1}	oral	14 days
Group 6	SDA-217B	6	125 mg kg^{-1}	oral	14 days
Group 7	SDA-217B	6	250 mg kg^{-1}	oral	14 days
Group 8	SDA-217B	6	500 mg kg^{-1}	oral	14 days

Sleep deprivation study

The animals were conditioned to sleep deprivation using a sleep deprivation apparatus, which is basically a motor-driven device, running bidirectionally at varying speeds according to a pre-programmed sequence during 12 h sleep deprivation experiments [26] (Table 5).

Drug administration was done orally at the beginning of the light cycle of the rats. Speed and the number of directional alternations were gradually increased over

time to compensate for increasing sleep pressure. The test drugs were given once per day as given in Table 6.

Sleep cycle measurements and monitoring were done by a closed-circuit camera [27] for 24 hr and after 12 hours of habituation to the sleep deprivation device, EEG was recorded using a PhysioPac instrument (Medicaid System, India), followed by 24 hours of baseline measurements and 12 hours of sleep deprivation [28]. Levels of plasma corticosterone and serotonin, norepinephrine, and dopamine of the brain (frontal and brain stem) were also estimated by ELISA kits [29, 30].

Table 5. Experimental protocol for conducting a sleep deprivation study

Study Protocol	Day 0	Day 1		Day 2		Day 3
Sleeping Time	**19:00-7:00**	**7:00-19:00**	**19:00-7:00**	**7:00-19:00**	**19:00-7:00**	**7:00-19:00**
Sleep deprivation	Hab	BL	BL	SD	Rec	Rec
EEG & biochemical tests	Hab	BL	BL	SD	Rec	Rec

Hab=habituation; BL=baseline; SD=sleep deprivation; Rec=recovery

Table 6. Experimental grouping of animals for conducting a Sleep deprivation study

Group	Test Drug	N	Dose	Route	Pretreatment
Group 1	Normal Control-Distilled water	6	5 ml kg^{-1}	oral	5 days
Group 2	Positive Control - Diazepam	6	4 mg kg^{-1}	oral	5 days
Group 3	SDA-217A	6	125 mg kg^{-1}	oral	5 days
Group 4	SDA-217A	6	250 mg kg^{-1}	oral	5 days
Group 5	SDA-217A	6	500 mg kg^{-1}	oral	5 days
Group 6	SDA-217B	6	125 mg kg^{-1}	oral	5 days
Group 7	SDA-217B	6	250 mg kg^{-1}	oral	5 days
Group 8	SDA-217B	6	500 mg kg^{-1}	oral	5 days

Cataleptic Study

Behavioural assessment in haloperidol-induced cataleptic rats was studied [31]. Cataleptic behaviour was measured with a high bar test method. The test drug was given as mentioned above. Positive control rats received haloperidol (3 mg kg^{-1}) intraperitoneally. Catalepsy score was measured at 1 h after test drug and haloperidol administration by gently placing both forepaws of the rat over a metal bar (diameter 2–5 mm suspended 6 cm above the table top). The intensity of catalepsy was assessed by counting the time in seconds until the rat brought both forepaws down to the tabletop, with a maximum cutoff time of 3 minutes.

Rotarod Study

The rotarod apparatus is mainly used for evaluating the skeletal muscle relaxing properties of rats and mice. In 1956, Dunham and Miya suggested that the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod [32]. The test compounds were administered orally. Zolpidem at the dose of 4 mg kg^{-1} oral dose was used as a prototype standard [33]. One hour after oral administration, the rats were placed for 1 min on the rotating rod. The number of animals falling from the roller during this time was counted.

Safety study (Irwin Test)

Irwin test or modified Irwin test, general neurobehavioral changes are observed along with other CNS actions. For assessing potential CNS effects, a functional observational battery (FOB) or similar test of general neurobehavioral changes, such as a modified Irwin test, is often used [34]. The test drug was given at the doses of 500 mg kg^{-1} and 1000 mg kg^{-1}, orally to mice and continuously monitored up to 4h.

The Behavioral Profile includes Awareness, Mood, and

Motor activity. The Neurological Profile includes CNS excitation, Posture, Muscle tone, and Reflexes. The Autonomic Profile includes respiratory rate, lacrimation, and mortality [35]. Effects of the drugs were observed and recorded on a scale of 0-8.

Hangover study

"Hangover" is featured by adverse physical and psychological problems that occur the morning following incomplete sleep or intake of excess doses of alcohol or sleeping pills. In the present study, two test drugs, SDA-217A and SDA-217B, were examined for a hangover study in mice. The animals were grouped and treated as follows for 14 days, as previously described. Thereafter, the animals were tested for activity in the cage. After 90 minutes of the last dose given, all animals were placed individually in the activity cage and the locomotor activity was assessed for a 10-minute period [36].

Statistical Analysis

The data were given in mean ± standard deviation. Percent change was calculated if necessary. The groups were statistically analyzed by analysis of variance and post hoc Dunnett's test using software (SPSS v.20). p-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

HPTLC fingerprint of test drugs

Two test drugs code-named SDA-217A and SDA-217B, were formulated and prepared by M/s Emami, Kolkata, one of the associate partners of the present ICMR project for pharmacological studies on hypnotic/sedative action in animals. A simple densitometric HPTLC chromatogram showed similarities of characteristic compounds and some differences also. The chromatograms are given in Figure 1.

Both test drugs SDA-217A and SDA-217B exhibited 7 characteristic bands, though the area percent of each band/peak varied between the two. These findings confirmed that both the test drugs have the same constituents but in different ratios. Quercetin was used as a biomarker. The standard chromatogram showed quercetin Rf at 0.37. Neither of the test drugs exhibited a peak for quercetin confirming its absence in the test drugs. The individual bands of peak area, and their respective Rf are given in Table 7. Peak 4 in test drug A (Rf 0.39) and peak 5 in test drug B (Rf 0.40) were quite similar, even in area % and also nearer to Rf 0.37 of biomarker quercetin.

Table 7. Comparison of the HPTLC chromatogram of the test drugs

Peak	Test Drug SDA-217A		Test Drug SDA-217B	
	Rf	Area (%)	Rf	Area (%)
1	0.00	19.92±0.004	0.00	16.57±0.006
2	0.15	5.53±0.006	0.15	4.59±0.003
3	0.33	5.52±0.001	0.25	3.21±0.001
4	0.39	14.46±0.008	0.32	5.50±0.003
5	0.48	2.57±0.009	0.40	15.54±0.007
6	0.50	2.58±0.002	0.50	5.08±0.003
7	0.71	25.77±0.006	0.70	28.24±0.006
8	0.81	23.95±0.008	0.81	21.28±0.005

Rf of quercetin=0.37; N=3; Mean ± SD

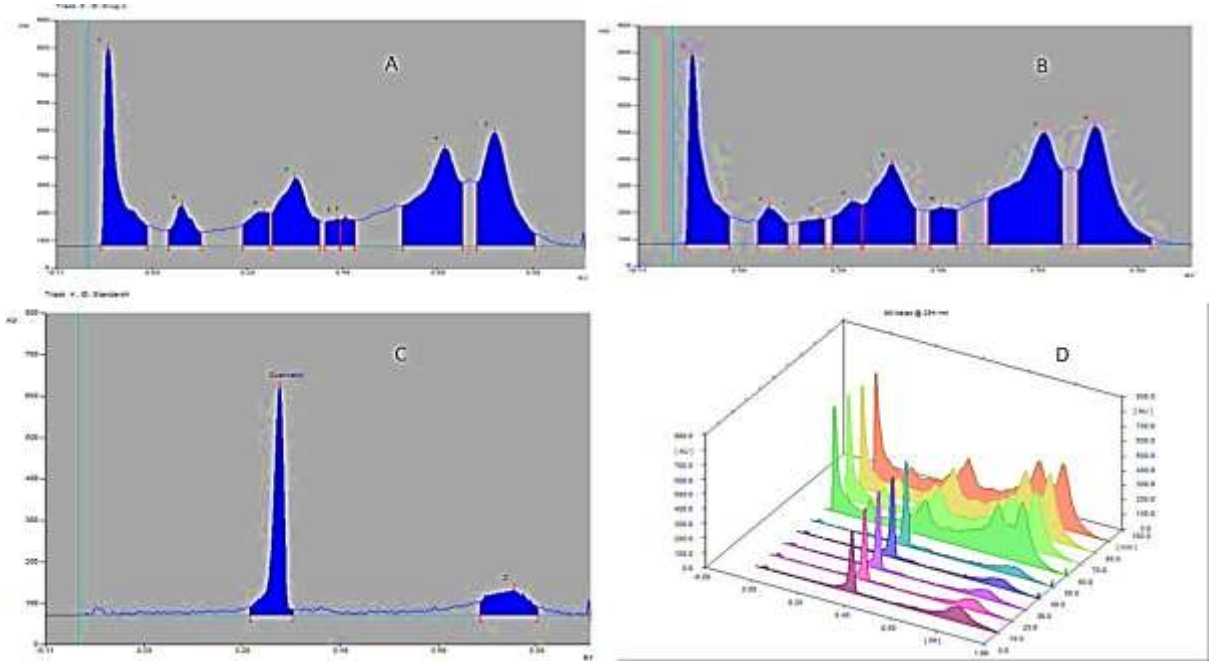

Figure 1. HPTLC chromatogram of A: SDA-217A, B: SDA-217B, C: Marker compound Quercetin, D: Comparison of peak area.

Pharmacokinetic Study

HPLC estimation of Withaferin A content in test drugs

SDA-217A and SDA-217B

In this study, Withaferin A present in *Withania somnifera* was estimated as a single marker. Previously developed HPLC method was used for estimation of Withaferin A. Withaferin A has been identified and estimated in both the test drugs SDA-217A and SDA-217B. In the test drug SDA-217A, the content of Withaferin A detected was found to be 36.98µg g^{-1} (Figure 2), and in SDA-217B, it was 107.38 µg g^{-1} (Figure 3).

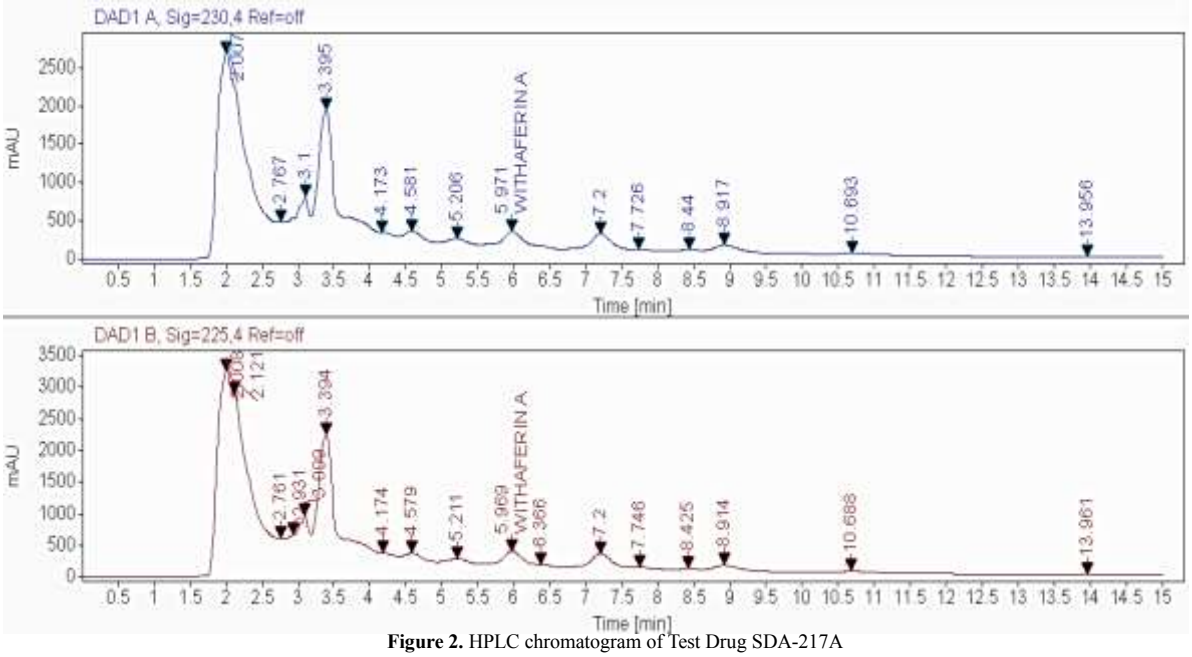

Figure 2. HPLC chromatogram of Test Drug SDA-217A

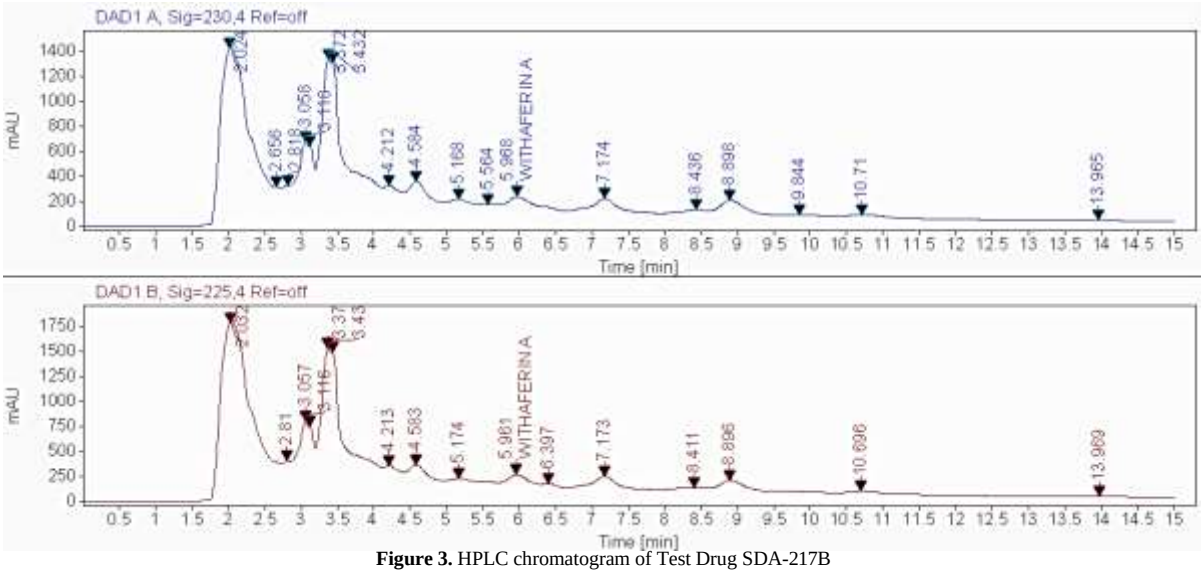

Figure 3. HPLC chromatogram of Test Drug SDA-217B

Bioavailability of test drugs (HPLC estimation of

Withaferin A in rat plasma)

Bioavailability of test drugs was measured by estimating plasma levels of Withaferin A and others in rat plasma obtained after administration of a single oral dose of 500 mg kg^{-1} of the test drugs SDA-217A and SDA-217B to overnight fasted rats. Blood was withdrawn at 0, 5, 10, 30, 60, and 120 minutes. An HPLC chromatogram was obtained for Withaferin A assessment. However, for both the test drugs SDA-217A (Figure 4) and SDA-217B (Figure 5), Withaferin A could be detected only in plasma samples obtained at 10 min, and it could not be detected at any other time points, which may be due to plasma levels below the detection limit. The standard HPLC curve of Withaferin A is given in Figure 6.

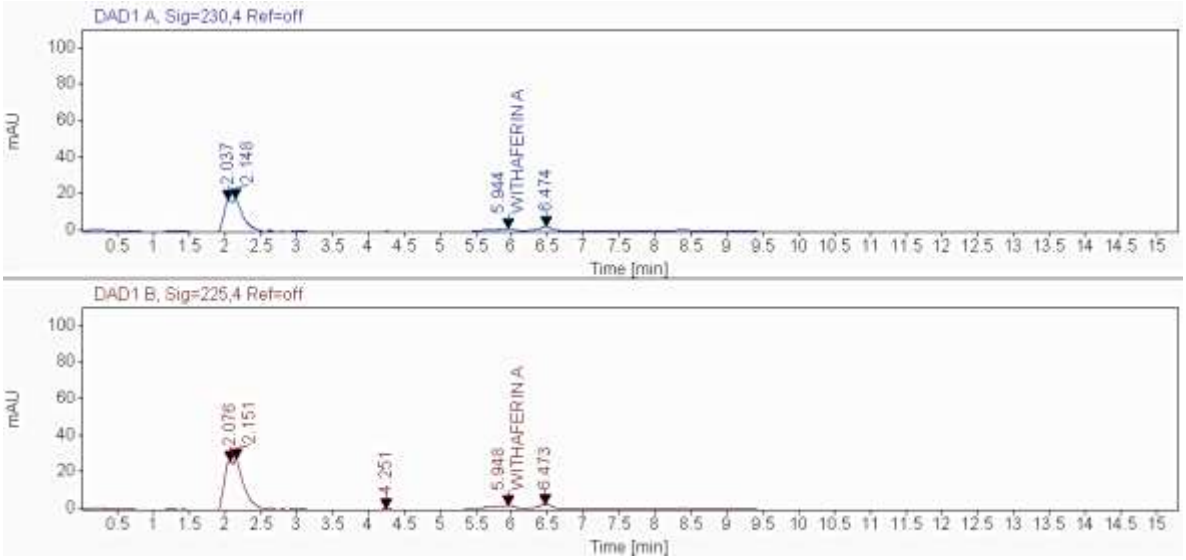

Figure 4. HPLC chromatogram of Test Drug SDA-217A in rat Plasma

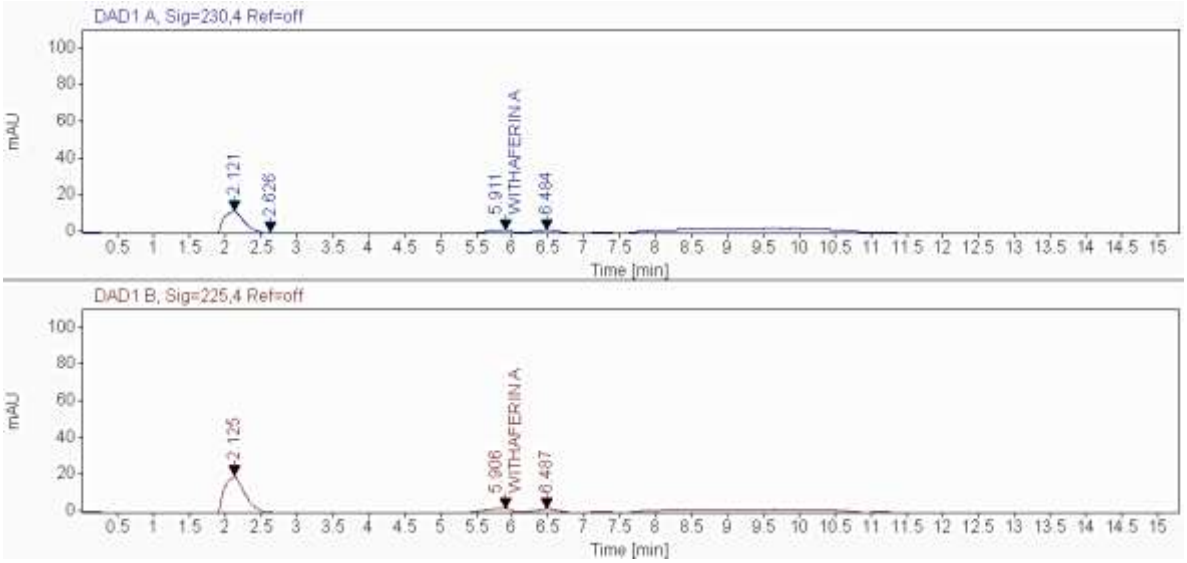

Figure 5. HPLC chromatogram of Test Drug SDA-217B in rat Plasma

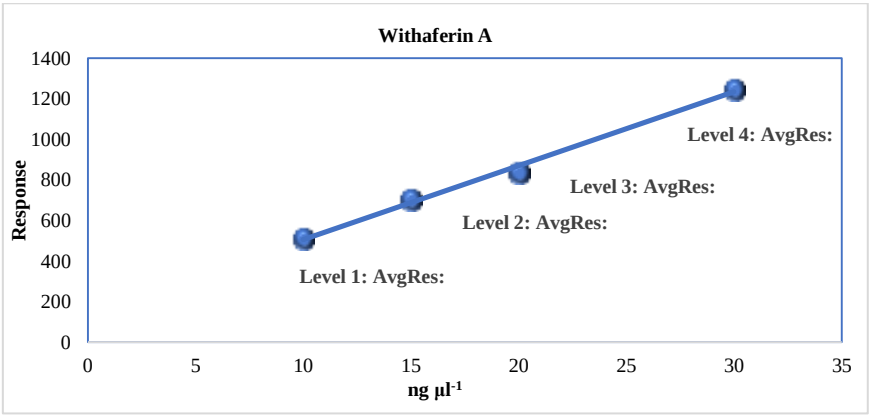

Figure 6. Standard curve of Withaferin A estimation by HPLC

Acute toxicity study

Neither of the test drugs showed any mortality in rats up to the single oral dose of 2.0 g kg^{-1} following the recommended guidelines. In higher doses, above 1.0 g kg^{-1}, the animals showed short-term drowsiness and restriction in somatomotor movement, but other CNS parameters were not found to be affected. Tremor, convulsions, and catalepsy were not seen in any animals after administration of test drugs, even at maximal administered doses of 2.0 g kg^{-1}, orally. Other reflexes, like reaction to touch, pupils, pinnal, corneal, righting etc., were also found quite similar to normal control animals up to the end of the study. Furthermore, excess salivation, any lacrimation, and diarrhea were not observed in any animal after drug treatments. The results are represented in Table 8.

The percentages of dead were corrected, and the probit values were also determined, but the 50% lethal doses (LD_{50}) could not be determined. The dose up to 2.0 g kg^{-1} orally in mice is safe and practically non-toxic. Extrapolation of results of acute oral toxicity/oral LD_{50} of animals is valid only to a very limited degree for humans. Based on the current results, the probable equivalent dose safe for humans is more than 10 g.

Table 8. Acute oral toxicity of test drugs SDA-217A and SDA-217B in albino mice

Test Drug: Group	Oral dose mg kg-1	Log dose	Dead / Total				Dead %	Corrected %	Probit
			Day 1	Day 2	Day 3	Day 14			
SDA-217A	500	2.69	0/3	0/3	0/3	0/3	0	8.33	3.61
	1000	3.0	0/3	0/3	0/3	0/3	0	8.33	3.61
	2000	3.30	0/3	0/3	0/3	0/3	0	8.33	3.61
SDA-217B	500	2.69	0/3	0/3	0/3	0/3	0	8.33	3.61
	1000	3.0	0/3	0/3	0/3	0/3	0	8.33	3.61
	2000	3.30	0/3	0/3	0/3	0/3	0	8.33	3.61

Corrected formula for 0%= 100(0.25/n), where n is the number of animals in the group

Hypnotic Study

Hypnotic potential of both test drugs i.e., SDA-217A and SDA-217B, was evaluated using 2 different methods.

Effect of test drugs on potentiation (onset of sleep) of thiopental sodium-induced sleeping time of rats

Test drug SDA-217A showed potentiation of sleep induction by 17.92%, 43.24%, and 54.16% at doses of 125 mg kg^{-1}, 250 mg kg^{-1}, and 500 mg kg^{-1} orally, respectively. Whereas, test drug SDA-217B at the same oral dose regimen potentiated sleep induction by 23.97%, 65.08%, and 75.47%, respectively (Table 9 and Figure 7).

Table 9. Effect of test drugs SDA-217A and SDA-217B on thiopental sodium-induced onset of sleep induction (loss of righting reflex) in Wistar rats

Groups	Treatment	Drug Code	Dose/oral	Onset of sleeping time (Sec)	change %
1	Control, DW	-	5 ml kg^{-1}	1350.60±82.37	-
2	Diazepam	Diaz	4 mg kg^{-1}	253.60±20.65*	-81.22
3	SDA-217A	A	125 mg kg^{-1}	1108.50±39.20*	-17.92
4	SDA-217A	A	250 mg kg^{-1}	766.50±31.17*	-43.24
5	SDA-217A	A	500 mg kg^{-1}	619.10±31.52*	-54.16
6	SDA-217B	B	125 mg kg^{-1}	1026.80±29.43*	-23.97
7	SDA-217B	B	250 mg kg^{-1}	471.60±36.20*	-65.08
8	SDA-217B	B	500 mg kg^{-1}	331.30±36.32*	-75.47

Results are Mean ± SEM; N=6; DW=distilled water; Statistical comparison was performed by ANOVA and followed by Post–hoc Dunnett's test; * indicates $p<0.01$; Percent change indicates the effect of test drugs on potentiation of sleep induction time after i/p injection of thiopental sodium in Wistar rats

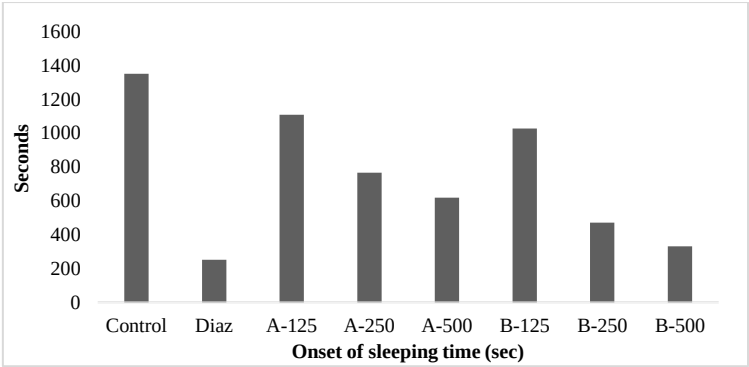

Figure 7. Effect of test drugs on thiopental sodium-induced loss of righting reflexes

Effect of test drugs on the duration of sleep in rats

Our present study exhibited (Table 10 and Figure 8) the outcome results on the duration of sleeping. The results indicate that the test drug SDA-217A at a similar dose has a better effect in enhancing the duration of sleep phenomenon or hypnotic action. Standard sedative drug diazepam (4 mg kg^{-1}, p.o.) enhanced sleeping time up by 241.33% compared to vehicle administered normal control The median dose of SDA-217A, *i.e.*, 250 mg kg^{-1}, enhanced the sleep duration by 189.29% compared to control, whereas at the same dose, test drug SDA-217B exhibited enhancement of sleep duration by 120.92% over control.

Table 10. Effect of test drugs SDA-217A and SDA-217B on thiopental sodium-induced duration of sleep in Wistar rats

Groups	Treatment	Dose/oral	Duration of sleeping (Sec)	% change
1	Control, DW	5 ml kg^{-1}	3162.83±129.88	-
2	Diazepam	4 mg kg^{-1}	10796±164.43*	+241.33
3	SDA-217A	125 mg kg^{-1}	5135.50±180.93*	+62.37
4	SDA-217A	250 mg kg^{-1}	9149.83±197.22*	+189.29
5	SDA-217A	500 mg kg^{-1}	11361.70±130.59*	+259.22
6	SDA-217B	125 mg kg^{-1}	4536.83±141.81*	+43.44
7	SDA-217B	250 mg kg^{-1}	6987.33±199.20*	+120.92
8	SDA-217B	500 mg kg^{-1}	9170.17±224.19*	+189.93

Results were Mean ± SEM; N=6; DW=distilled water; Statistical comparison was performed by ANOVA and followed by Post–hoc Dunnett's test; * indicates $p<0.01$; Percent change indicates potentiation of test drugs on inducing sleeping time after i/p injection of thiopental sodium (hexobarbital) in Wistar rats.

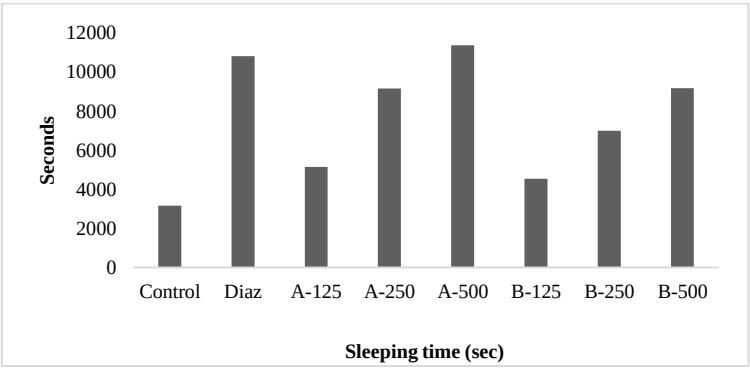

Figure 8. Effect of test drugs SDA-217A and SDA-217B on potentiation of thiopental sodium-induced hypnotic action (duration of sleep)

Depression Study

Sleep deprivation is one of the important causes of depression. So, the results of the Depression studies, like Porsolt's forced swimming test, are as follows.

Porsolt's forced swimming test

In this study, positive control, Imipramine, lowered depression scores up to 44.05% in mice (Table 11 and Figure 9). Both the test drugs showed dose-dependent anti-depressant properties. Moreover, the pharmacological action of drug SDA 217A and drug SDA 217B was the same. At the dose of 250 mg kg^{-1}, drug SDA 217A reduced the depression score by 34.93% and drug SDA 217B by 34.38%.

Table 11. Effect of test drugs SDA-217A and SDA-217B on forced swim-induced depression in mice.

Groups	Treatment	Dose/Oral	Depression score immobility time (Sec)	% Change
1	Control, DW	5 ml kg^{-1}	211.83±8.61	-
2	Imipramine	15 mg kg^{-1}	118.50±7.97*	-44.05
3	SDA-217A	125 mg kg^{-1}	179.50±8.14*	-15.26
4	SDA-217A	250 mg kg^{-1}	151.60±7.39*	-28.43
5	SDA-217A	500 mg kg^{-1}	137.83±6.96*	-34.93
6	SDA-217A	125 mg kg^{-1}	185.83±8.78*	-12.27
7	SDA-217B	250 mg kg^{-1}	154.33±9.17*	-27.14
8	SDA-217B	500 mg kg^{-1}	139±7.21*	-34.38

Results were Mean ± SEM; N=6; S DW=distilled water; statistical comparison was performed by ANOVA and followed by Post–hoc Dunnett's test; * indicates $p<0.01$; Percent change indicates potentiation of test drugs on depression score or immobility time (sec) on Swim-Test in mice.

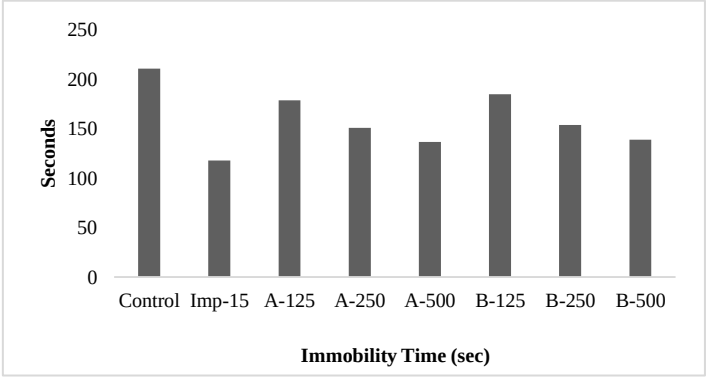

Figure 9. Depression scores in mice after treatment with test drugs SDA-217A and SDA-217B

Antioxidant parameters

In the present study on swim stress-mediated depression in mice, enhanced the lipid peroxidation in brain tissues by more than 147%, and reduced the brain GSH by80%, SOD activity by 48% and nitric oxide levels by 73%. Antidepressant drug Imipramine lowered the lipid peroxidation by 21% and improved the SOD activity by 27%, GSH levels by more than 4-fold, and NO levels by over 50%. Interestingly, both test drugs SDA-217A and SDA-217B showed significant antioxidant effects. Test drug SDA 217B showed 34% inhibition in lipid peroxidation in the brain compared to 25% of test drug SDA 217 A. SDA 217B also significantly enhanced the reduced brain GSH level by 332%, SOD activity by 68.75% and nitric oxide levels by 97% (Table 12).

Table 12. Effect of test drugs SDA-217A and SDA-217B on oxidative and anti-oxidative markers in the brain of forced swim-induced depression in mice.

Treatment	Oral Dose	LPO (µg/mg protein)	SOD (U/mg protein)	GSH (mM/mg protein)	NO (µM/mg protein)
Normal Control, DW	5 ml kg^{-1}	0.021±0.008	0.31±0.003	2.15±0.08	1.47±0.08
Swim Control, DW	5 ml kg^{-1}	0.052±0.006(a)*	0.16±0.005(a)*	0.43±0.04(a)*	0.39±0.04(a)*
Imipramine	15 mg kg^{-1}	0.041±0.004(b)*	0.22±0.008(b)*	1.95±0.06(b)*	0.59±0.01(b)*
SDA-217A	125 mg kg^{-1}	0.049±0.008(b)*	0.19±0.009(b)*	0.98±0.09(b)*	0.41±0.03(b)*
SDA-217A	250 mg kg^{-1}	0.042±0.007(b)*	0.20±0.007(b)*	1.35±0.04(b)*	0.44±0.02(b)*
SDA-217A	500 mg kg^{-1}	0.039±0.004(b)*	0.24±0.002(b)*	1.72±0.05(b)*	0.46±0.02(b)*
SDA-217B	125 mg kg^{-1}	0.046±0.004(b)*	0.20±0.006(b)*	1.12±0.07(b)*	0.43±0.04(b)*
SDA-217B	250 mg kg^{-1}	0.039±0.005(b)*	0.22±0.004(b)*	1.69±0.03(b)*	0.62±0.01(b)*
SDA-217B	500 mg kg^{-1}	0.034±0.002(b)*	0.27±0.001(b)*	1.86±0.09(b)*	0.77±0.08(b)*

Results were Mean ± SEM; N=6; DW=Distilled water; LPO= lipid peroxides; SOD= superoxidedismutase; GSH= reduced glutathione; NO= nitric oxides; Statistical comparison was performed by ANOVA and followed by Post–hoc Dunnett's test; * indicates $p<0.01$; Normal Control mean without Swim; Normal (a) compared to Swim control (b); Swim Control (b) compared with other treated drugs.

Plasma corticosterone levels in mice

In this study, forced swim induced the plasma corticosterone enhancement up to 134%, which was reduced by Imipramine and test drugs SDA-217A and SDA-217B treatments. SDA-217B reduced plasma corticosterone levels by 31.72%.

Brain neurotransmitter levels

In this study, forced swim despair in mice increased their brain DA levels by 35%, NE levels by 56%, and 5-HT levels by 83%. The imipramine treatment significantly blocked the rise in brain DA, NE and 5HT levels. Imipramine treatment group showed a rise in levels of DA by 6.6%, NE levels by 14.2% and 5-HT levels by 6.8% proving its antidepressant action (Table 9). Interestingly, both the test drugs also showed dose-dependent neurotransmitter-modulating properties. Both test drugs SDA-217A and SDA-217B inhibited the rise in brain DA, NE, and 5HT levels, which was nearly comparable to imipramine. A compiled table of all the neurotransmitter levels before and after the experiment, with graphical representation, has been represented in Table 13 and Figure 10, respectively.

Table 13. Effect of test drugs SDA-217A and SDA-217B on central neurotransmitters with depression mice.

Treatment	Dose/Oral	Corticosterone (ng/ml)	DA (ng/mg protein)	NE (pg/ mg protein)	5-HT (ng/ mg protein)
Normal Control, DW	5 ml kg^{-1}	9.28±0.65	318.33±12.36	203.52±16.97	29.56±0.94
Swim Control, DW	5 ml kg^{-1}	21.75±1.28(a)*	430.45±18.64(a)*	318.50±14.52(a)*	54.22±1.82(a)*
Imipramine	15 mg kg^{-1}	12.16±0.86(b)*	339.85±10.97(b)*	232.17±15.84(b)*	31.55±0.75(b)*
SDA-217A	125 mg kg^{-1}	16.05±0.74(b)*	392.15±12.44(b)*	269.88±17.26(b)*	45.08±1.15(b)*
SDA-217A	250 mg kg^{-1}	14.24±0.39(b)*	369.08±11.68(b)*	243.57±14.24(b)*	43.89±1.08(b)*
SDA-217A	500 mg kg^{-1}	13.73±0.85(b)*	340.75±9.92(b)*	221.50±10.82(b)*	38.74±0.98(b)*
SDA-217B	125 mg kg^{-1}	17.59±0.72(b)*	371.85±10.45(b)*	234.95±15.68(b)*	36.05±1.16(b)*
SDA-217B	250 mg kg^{-1}	15.62±0.91(b)*	338.25±9.68(b)*	212.68±12.08(b)*	31.15±1.22(b)*
SDA-217B	500 mg kg^{-1}	14.85±0.55(b)*	305±9.15(b)*	208.83±13.65(b)*	30.92±1.15(b)*

Results were Mean ± SEM; N=6; DW=distilled water; Statistical comparison was performed by ANOVA and followed by Post–hoc Dunnett's test; * indicates $p<0.01$; Normal Control mean without Swim; Normal (a) compared to Swim control (b) and Swim Control (b) compared with other treated drugs

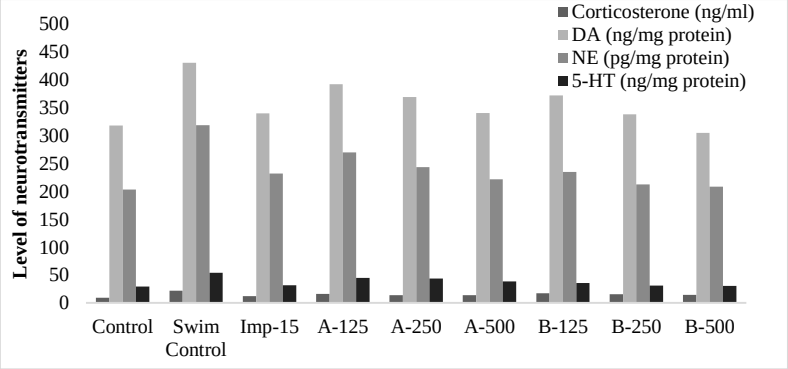

Figure 10. Neurotransmitter levels in mice after treatment with test drugs SDA-217A and SDA-217B

Anxiety study

Anxiety and depression are also commonly linked with sleep deprivation, which can lead to feelings of hopelessness and worthlessness that last for long periods of time. To induce anxiety in the animals and hence test the anxiolytic effect of the test drugs, some models were used as follows.

Chronic foot-shock model

In this study, chronic foot-shock produced fear responses and anxiety in rats and due to anxiousness, open arm entries, and time spent in the open arm were declined (Table 14). Treatment with both test drugs SDA-217A & SDA-217B and diazepam exhibited a significant increase in both the number of entries and time spent in the open arms, while the number of entries and time spent in the closed arms declined compared to vehicle-treated chronic foot-shock-induced control rats. The standard anxiolytic drug diazepam showed the highest effects amongst all (Table 14).

Table 14. Effect of test drugs SDA-217A and SDA-217B on foot-shock-induced anxiety in Wistar rats.

Group	Treatment	Open arm (Sec)	Closed arm (Sec)	Number of entries in the open arm
1	Control, DW-5 ml kg^{-1}	45±5.62	241.33±7.96	3.83±0.98
2	Diaz- 4mg kg^{-1}	107.1±8.51*	177.50±4.50*	10.33±1.03*
3	SDA-217A-125mg kg^{-1}	57.80±3.76*	232.16±4.02*	6.33±1.36*
4	SDA-217A-250mg kg^{-1}	75±3.89*	216.16±3.71*	7.50±1.05*
5	SDA-217A-500mg kg^{-1}	89.30±7.73*	197.50±7.81*	9.50±1.04*
6	SDA-217B-125mg kg^{-1}	54±4.09*	234.33±5.95*	6.66±1.21*
7	SDA-217B-250mg kg^{-1}	71.50±2.42*	211.83±4.70*	7.83±0.75*
8	SDA-217B-500mg kg^{-1}	85.10±5.92*	198.33±7.08*	9.83±1.32*

Results were Mean ± SEM; N=6; Diaz= diazepam; DW=distilled water; Statistical comparison was performed by ANOVA and followed by Post–hoc Dunnett's test; * indicates $p<0.01$; Test drugs on foot-shock-induced anxiety score in Wistar rats.

Brain Antioxidant parameters

Foot-shock induced anxiety raised the brain LPO levels by 186% and diminished the GSH levels by 49%, SOD activity by65% and NO levels by 74% in rats' brain (Table 15). The modern anxiolytic diazepam lowered anxiety by protecting against oxidative damage in the brain, as presented in Table 10. Diazepam treatment inhibited the LPO by 74%, enhanced the GSH levels by 6.8%, SOD activity by 9% and NO levels by 75%. Interestingly, test drug SDA-217B showed inhibition in LPO by 72%, enhanced the GSH levels by

77%, SOD activity by 95% and NO release by 160%.

Table 15. Effect of test drugs SDA-217A and SDA-217B on markers of oxidative stress in foot-shock-induced anxiety rats

Treatment	Oral Dose	LPO (µg/mg protein)	SOD (U/mg protein)	GSH (mM/mg protein)	NO (µM/mg protein)
Normal Control, DW	5 ml kg^{-1}	0.015±0.009	0.66±0.036	2.03±0.02	1.97±0.035
Stress Control, DW	5 ml kg^{-1}	0.043±0.013(a)*	0.23±0.034(a)*	1.02±0.04(a)*	0.51±0.032(a)*
Diazepam	4 mg kg^{-1}	0.011±0.001(b)*	0.27±0.046(b)*	1.09±0.08(b)*	0.89±0.060(b)*
SDA-217A	125 mg kg^{-1}	0.031±0.007(b)*	0.31±0.081(b)*	1.18±0.03(b)*	1.59±0.073(b)*
SDA-217A	250 mg kg^{-1}	0.022±0.005(b)*	0.39±0.063(b)*	1.21±0.02(b)*	1.75±0.099(b)*
SDA-217A	500 mg kg^{-1}	0.016±0.004(b)*	0.45±0.074(b)*	1.32±0.02(b)*	1.94±0.038(b)*
SDA-217B	125 mg kg^{-1}	0.025±0.006(b)*	0.35±0.044(b)*	1.31±0.05(b)*	1.79±0.036(b)*
SDA-217B	250 mg kg^{-1}	0.019±0.008(b)*	0.42±0.054(b)*	1.38±0.04(b)*	2.04±0.085(b)*
SDA-217B	500 mg kg^{-1}	0.012±0.002(b)*	0.48±0.029(b)*	1.81±0.03(b)*	2.32±0.026(b)*

Results were Mean ± SEM; N=6; DW=distilled water; LPO= lipid peroxides; SOD= superoxide dismutase; GSH= reduced glutathione; NO= nitric oxides; Statistical comparison was performed by ANOVA and followed by Post–hoc Dunnett's test; * indicates $p<0.01$; Normal Control mean without Stress; (a) compared to normal control (b); compared to stress control.

Plasma corticosterone and central (brain) neurotransmitter levels

In this study, foot-shock stress elevated the plasma corticosterone levels by more than 95%. Foot-shock stress also enhanced central DA levels by 84%, NE levels by 83% and 5-HT levels by 73% compared to normal control (Table 16). Both the test drugs exhibited significant anxiolytic properties, which are comparable to diazepam; however, SDA-217B at the dose of 500 mg kg^{-1}, p.o., showed better anxiolytic effect than the same dose of SDA-217A. Treatment with SDA-217B at the dose of 500 mg kg^{-1}, p.o., also inhibited the plasma corticosterone surge by 35% and attenuated brain neurotransmitter levels of DA (by 35%), NE (by 38%) and 5-HT (by 50%).

Table 16. Effect of test drugs SDA-217A and SDA-217B on central (brain) neurotransmitters levels in foot-shock-induced anxiety rats.

Treatment	Oral dose	Corticosterone (ng/ml)	DA (ng/mg protein)	NE (pg/ mg protein)	5-HT (ng/ mg protein)
Normal Control, DW	5 ml kg^{-1}	19.62±1.28	315±8.65	216.50±18.28	26.89±0.89
Swim	5 ml kg^{-1}	38.45±2.14(a)*	397.72±9.12(a)*	397.66±20.35(a)*	46.67±1.75(a)*
Control, DW Diazepam	4 mg kg^{-1}	23.08±1.96(b)*	252.27±7.44(b)*	248.32±16.97(b)*	22.11±0.82(b)*
SDA-217A	125 mg kg^{-1}	32.22±1.48(b)*	301.15±10.27(b)*	295.16±22.84(b)*	37.95±1.11(b)*
SDA-217A	250 mg kg^{-1}	29.64±1.22(b)*	284.65±9.35(b)*	281.75±19.62(b)*	33.46±1.34(b)*
SDA-217A	500 mg kg^{-1}	26.15±1.39(b)*	273.41±8.33(b)*	263.50±19.54(b)*	29.33±1.25(b)*
SDA-217B	125 mg kg^{-1}	28.32±1.68(b)*	286.55±9.17(b)*	264.08±22.15(b)*	31.09±1.08(b)*
SDA-217B	250 mg kg^{-1}	26.15±1.74(b)*	267.94±8.94(b)*	256.92±21.75(b)*	27.15±1.65(b)*
SDA-217B	500 mg kg^{-1}	24.86±1.26(b)*	258.03±9.55(b)*	243.50±18.63(b)*	23.33±1.15(b)*

Results were Mean ± SEM; N=6; DW=distilled water; Statistical comparison was performed by ANOVA and followed by Post–hoc Dunnett's test; * indicates $p<0.01$; Normal Control mean without Stress; Normal (a) compared to Stress control (b); Stress Control (b) compared with other treated drugs

Sleep Deprivation Study

A self-designed sleep deprivation study instrument (videography recording) has been prepared and validated for the present study.

Sleep duration in sleep-deprived rats

In the present sleep deprivation study, normal rats slept for a duration of 10-11 h; whereas sleep-deprived rats slept only for 1-2 h. Rats treated with test and reference drugs showed improvement in their sleeping patterns. Diazepam improved the 12 hours' sleep deprivation period over control animals by 295%. Treatments with SDA-217A and SDA-217B at the dose of 500 mg kg^{-1}, p.o., showed significant improvement in sleep duration of sleep-deprived animals by 238% and 244%, respectively (Table 17).

Table 17. Effect of test drugs SDA-217A and SDA-217B on sleep duration in sleep-deprived rats

Treatment	Dose/Oral	Sleep duration (Minutes)		
		Day 1 (Habituation)	Day 2 (Sleep Deprivation)	Day 3 (Recovery)
Normal Control, DW	5 ml kg^{-1}	642±10.65	656±12.54	672±14.15
Deprived Control, DW	5 ml kg^{-1}	248±12.83(a)*	68±8.64(a)*	298±12.82(a)*
Diazepam	4 mg kg^{-1}	478±9.64(b)*	269±8.59(b)*	515±11.64(b)*
SDA-217A	125 mg kg^{-1}	379±10.22(b)*	189±7.35(b)*	410±12.03(b)*
SDA-217A	250 mg kg^{-1}	398±11.73(b)*	210±9.28(b)*	456±14.08(b)*
SDA-217A	500 mg kg^{-1}	416±12.42(b)*	230±10.24(b)*	486±11.16(b)*
SDA-217B	125 mg kg^{-1}	395±10.84(b)*	175±8.25(b)*	428±12.43(b)*
SDA-217B	250 mg kg^{-1}	410±8.94(b)*	188±11.14(b)*	471±11.87(b)*
SDA-217B	500 mg kg^{-1}	424±10.45(b)*	234±9.52(b)*	494±10.57(b)*

Results were Mean ± SEM; N=6; Unit=minutes; DW=distilled water; Statistical comparison was performed by ANOVA and followed by Post–hoc Dunnett's test; * indicates $p<0.01$; Normal Control mean without sleep deprivation; Normal (a) compared to sleep deprivation control (b); sleep deprivation control (b) compared with other treated drugs.

Electrophysiological Study

Unfortunately, the results of the electrophysiological study in conscious rats were varied from animal to animal and also in the same and different groups. The output EEG recordings of animals' experiments designed in the sleep-deprived rat model were not reproducible and significant.

Plasma corticosterone and central (brain) neurotransmitter levels

Plasma level of corticosterone was raised (by 115%) and levels of three important neurotransmitters of the brain were significantly elevated in sleep-deprived animals i.e., dopamine (DA by 80.9%), norepinephrine (NE by 61.4%), and serotonin (5-HT by 91.9%) (Table 18). Diazepam treatment significantly prevented this rise in the levels of plasma corticosterone and levels of brain DA, NE, and 5-HT, and the rise in these levels compared to normal control is 40.8%, 30%, 30.1% and 6.6%, respectively. Both the test drugs, SDA-217A and SDA-217B, also significantly inhibited the rise in plasma levels of corticosterone and neurotransmitter levels in the brain in a dose-dependent manner, which was found comparable to diazepam. SDA-217A treatment at the dose of 500 mg kg^{-1} resulted in a rise of plasma corticosterone level by 53.6%, brain DA level by 38.8%, brain NE level by 36% and brain 5-HT level by 16.8%. SDA-217B treatment afforded this rise of plasma corticosterone level by 50.9%, brain DA level by 21.3%, brain NE level by 25.2 % and brain 5-HT level by 13.2%.

Table 18. Effect of test drugs on central neurotransmitters in sleep-deprived rats

Treatment	Oral dose	Corticosterone (ng/ml)	DA (ng/mg protein)	NE (pg/ mg protein)	5-HT (ng/ mg protein)
Normal Control, DW	5 ml kg^{-1}	19.62±1.28	215±7.12	216.50±18.28	26.89±0.89
Sleep deprived Control, DW	5 ml kg^{-1}	42.27±2.15(a)*	389.09±10.24(a)*	349.65±26.58(a)*	51.62±1.19(a)*
Diazepam	4 ml kg^{-1}	27.64±1.69(b)*	279.55±8.56(b)*	281.75±18.37(b)*	28.67±0.85(b)*
SDA-217A	125 ml kg^{-1}	36.89±1.56(b)*	324.16±7.11(b)*	314.66±20.14(b)*	38.94±1.35(b)*
SDA-217A	250 ml kg^{-1}	32.55±1.88(b)*	311.97±10.86(b)*	303.98±19.12(b)*	34.75±1.22(b)*
SDA-217A	500 ml kg^{-1}	30.15±1.25(b)*	298.63±9.07(b)*	294.68±20.89(b)*	31.42±1.49(b)*
SDA-217B	125 ml kg^{-1}	35.62±1.39(b)*	309.74±11.25(b)*	305.12±24.64(b)*	36.55±1.24(b)*
SDA-217B	250 ml kg^{-1}	31.14±1.57(b)*	280.12±10.42(b)*	287.06±22.42(b)*	33.19±1.08(b)*
SDA-217B	500 ml kg^{-1}	29.62±1.38(b)*	261.82±9.86(b)*	271.25±18.97(b)*	30.45±1.15(b)*

Results were Mean ± SEM; N=6; DW=distilled water; Statistical comparison was performed by ANOVA and followed by Post–hoc Dunnett's test; * indicates $p<0.01$; Normal Control mean without sleep deprivation Normal (a) compared to sleep deprivation control (b); sleep deprivation control (b) compared with other treated drugs.

Catalepsy Study

Catalepsy was observed when rats were placed in abnormal or unusual postures 1hour after haloperidol (3 mg kg^{-1}, i.p.) administration, and these animals maintained these postures for a period of time (3 minutes). A normal animal corrects its position within seconds and explores its environment, but a cataleptic animal maintains this externally imposed posture for a prolonged period of time. Standard drug haloperidol induced catalepsy in all animals; whereas in the drug treated group, all animals showed normal posture, and no drug-related narcolepsy or catalepsy was observed.

Motor in-coordination study

The Rota-rod test was used to study the effects of test drugs on motor coordination. Treatment with test drugs (SDA-217A and SDA-217B) up to 500 mg kg^{-1} oral doses did not show any muscle incoordination activity in rats, within the cut-off time of one minute (2 rotations/min), as no animal fell from the rotating rod within the stipulated time.

Hangover Study

The animals were grouped and treated as described previously in the methodology section and followed for 14 days. Thereafter, the animals were tested for locomotor behavior in an activity cage. After 90 minutes of the last dose administration, all animals were placed individually in the activity cage and the locomotor activity was assessed for a 10-minute period (Table 19). There were no statistically significant differences seen in the locomotor activity of animals between different groups.

Table 19. Effect of test drugs (SDA-217A and SDA-217B) on locomotor activity of rats

Treatment	Dose/Oral	Locomotor activity (score)
Normal Control, DW	5 ml kg^{-1}	339.83±13.45
Diazepam	4 ml kg^{-1}	339±17.36
SDA-217A	125 ml kg^{-1}	330.83±18.02
SDA-217A	250 ml kg^{-1}	333±14.59
SDA-217A	500 ml kg^{-1}	324.16±9.91
SDA-217B	125 ml kg^{-1}	328.66±19.86
SDA-217B	250 ml kg^{-1}	322±18.91
SDA-217B	500 ml kg^{-1}	327±22.80

Results were Mean ± SEM; N=6; DW=distilled water; Statistical comparisons were performed by ANOVA and followed by Post–hoc Dunnett's test; * indicates $p<0.01$; all data compared to Normal control; all data were statistically not significant.

Safety Study

The Irwin test in rodents assesses the effects of test drugs on behavior, physiology, and safety. Here, the Irwin test was performed to assess the CNS safety pharmacology of two test drugs (SDA-217A and SDA-217B), at two oral doses of 500 mg kg^{-1} and 1000 mg kg^{-1} in mice. The observations from the study were mild sedation in between 1 to 2 hours, post administration of both the formulations. Furthermore, some other effects like decreased fear, analgesia, ptosis, exophthalmia, myosis, mydriasis, piloerection, salivation, and lacrimation were observed 4 hours post administration of the test drugs to a very small extent, establishing the safety profile of both drugs.

CONCLUSIONS

From the HPLC findings, it might be concluded that both the test drugs bear a similar type of constituents, but the ratio of the phytoconstituents present in them varies from one another. From the Pharmacokinetic study performed, it can be concluded that the rate of absorption of both the test drugs is rapid with respect to Withaferin A content and therefore, both the test drugs, SDA-217A and SDA-217B, is orally bioavailable and can be developed as drugs.

Regarding the safety of both test drugs, the Acute toxicity study was performed. From the results of that test, it could be concluded that both the test drugs, SDA-217A and SDA-217B, did not possess any toxicity nor had any lethal effects up to the tested doses and are safe for oral use.

Although SDA-217B produced better results in the onset of sleep experiment, but SDA-217A exhibited more potent effect in enhancing the duration of sleep than the other test drug. Overall, the above results of the depression study are suggestive of having significant antidepressant activities in both the test drugs; however, the antioxidant and neurochemical modulating effects of SDA-217B are slightly better compared to SDA-217A. From the anxiety study, it could be concluded that both the test drugs have a very good anxiolytic effect based upon the results.

Both test drugs SDA-217A and SDA-217B showed promising beneficial effects on sleep enhancement, reduction in plasma corticosterone levels, and modulation of central neurotransmitters of sleep-deprived animals in a positive manner. Hence, hypnotic effects observed for these drugs might be therapeutically useful for sleep disorders in humans. Similarly, any cataleptic or narcoleptic effects were absent in test animals. No muscle relaxing properties of the test drugs were observed when they were used up to the therapeutic doses. Post-use of the test drugs, no hangover-like effect was observed, and all the animals showed normal locomotor behaviour.

From the battery of behavioural and physiological observations, neither of the test drugs produced any significant alterations except the expected sedative-related effects in mice. Therefore, it may be concluded that both the test drugs are CNS safe at least up to 1000 mg kg^{-1}, orally in mice.

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ETHICAL CONSIDERATION

The Institutional Animal Ethical Committee of 'R.G. Kar Medical College' has approved this work. The Approval number is 'RKC/IAEC/14/14, dated 7.9.2017'.

Conflict of interests

There is no conflict between the authors to publish this article to your esteemed journal, "Journal of Chemical Health Risks".

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Author contribution

Dr. Anjan Adhikari made the idea of the research and

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Availability of data and material

The work was performed in the laboratories of R.G. Kar Medical College, Kolkata, and West Bengal, India. The information regarding the methods was collected from the various sources of reputed journals.

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