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SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL SCREENING OF SCHIFF-BASES THROUGH STRUCTURE ACTIVITY RELATIONSHIP (SAR) STUDY IN MICE

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ABSTRACT

Background: Three benzaldehyde derived Schiff-bases (**SH 1-3**) were synthesized by the condensation reaction from *o*-phenylenediamine with their respective benzaldehyde derivatives in absolute ethanolic solution under reflux reaction mixture for 6-8 hrs. The structural determinations of the synthesized Schiff-bases were confirmed by FT-IR and ^{1}H-NMR spectroscopy. Benzaldehyde derived Schiff-bases are well-known to possess potent antioxidant, anxiolytic and sedative potential activities etc. In view of the importance of various benzaldehyde derivatives, the present investigation was undertaken which deals with the structure activity relationship (SAR) study and evaluation of the antioxidant, anxiolytic and sedative potential activities of synthesized Schiff-bases in mice models.

Materials and Methods: ABTS scavenging activity was used to assess the antioxidant activity. Acute toxicity test was attained to determine the toxic dose and optimum therapeutic dose. Elevated plus maze model, Light dark model and Hole board test were performed to investigate the anxiolytic effects, while Thiopental induced sleeping time test was executed for the sedative potential ability of synthesized compounds.

Results: In SAR study, the results revealed that the synthesized compounds **SH 3**, **SH 2** and **SH 1** at dose of 50 mg/kg show potent pharmacological activities like antioxidant, anxiolytic and sedative potential activities respectively as compared to the control group at ($p<0.001$).

Conclusion: The compound **SH 1** was found to possess poor pharmacological effects while the compounds **SH 2** and **SH 3** showed significant pharmacological results which prompted us to study their other pharmacological activities like antiviral, antibacterial, anticancer and so on. Also, a relationship was showed between the CNS depressant effect of synthesized compounds and diazepam since further studies are require to exposed the underlying mechanism in detail.

INTRODUCTION

An organic compound having an azomethine group (−HC═N−) is known as Schiff base. Schiff bases have been reported for the first time by Hugo Schiff in 1864. Schiff bases are produced by the condensation of primary amine with an aldehyde or ketone (More, Raut et al. 2017). Benzaldehydes are the essential class of organic compounds and it has been stated that metal complexes of benzaldehyde Schiff bases have enormous applications and act as key intermediates in the synthesis of different heterocyclic compounds (Kumari, Shekhar et al. 2016). The main cause of oxidative damages in the body are the unpaired electrons of the free radicals that promotes diabetes mellitus, cancer and heart diseases. Antioxidants have the capability to accept or donate electrons hence they inhibit oxidation of molecules (Retnaningtyas and Setiadi 2017). Sankarganesh et al., (2017) synthesized Schiff bases derivatives and screen for their potent antioxidant activity (Saleem, Sankarganesh et al. 2017). Anxiety is a common psychological and physiological condition involving tension, enhanced sympathetic action and nervousness etc. Synthetic anxiolytic drugs such as benzodiazepines, diazepam and buspirone have been employed for the treatment of anxiety disorders (Mahendran, Thamotharan et al. 2014). Various Schiff bases derivatives i.e. quinazolinone derivatives (Gavin, Annor-Gyamfi et al. 2018), isatin Schiff bases (Jaiswal, Tripathi et al. 2018) and a series of thiosemicarbazone derivatives (Cavusoglu, Saglik et al. 2018) possess significant anxiolytic properties. Sedative hypnotic activity has been exhibited by oxadiazoles derived schiff bases (Taha and Rasheed 2018). In view of the importance of various benzaldehyde derivatives, in this document we discuss synthesis of benzaldehyde derived Schiff bases and their potent antioxidant, acute toxicity, anxiolytic and sedative potential activities.

Materials and Methods

Chemicals and materials

Different solvents used in my research are distilled water, methanol, normal saline and Tween 80 that's were purchased in Lahore (Pakistan) from Merck. The reactants such as *o*-phenylenediamine and aldehyde derivatives i.e. 4-nitrobenzaldehyde, 4-chlorobenzaldehyde and 4-dimethylbenzaldehyde were accepted as a kind gift from Chemistry Department at University of Azad Jammu and Kashmir. Diazepam (10 mg/2 ml) and Thiopental sodium (Pentothal 500 mg) were also used.

Instrumentation

The compounds were confirmed by ^{1}H-NMR spectra at 300 MHz through Bruker Avance spectrometer with an internal standard used as TMS (titramethyl silane). Parts per million (ppm) was recorded to point out the chemical shift values. Also IR Spectroscopy was obtained, for IR spectra Bio-Rad Merlin Fourier Transform Infra-Red spectrophotometer was used. Melting points of synthesized compounds were got in Barnstead electrothermal apparatus. TLC plates (Merck 60 F_{254}) procured from Merck Darmstadt Germany were used to observed product formation under UV lamp.

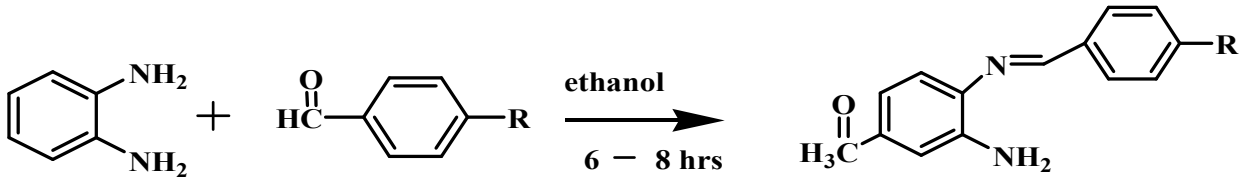
Animals used

Male albino mice weighting (20-25 grams) were procured from National Institute of Health Islamabad Pakistan. Pieces of soft wood like bedding were used to kept mice in cages and acclimatization period was provided under standard laboratory conditions. Animals were placed in a room having 12 hrs of light and dark cycle at control temperature and standard diet was supplied on the daily need base.

Methodology

Synthesis of Schiff bases (SH 1–3)

The synthesized benzaldehyde derived Schiff bases (**SH 1–3**) were prepared according to the reported protocols. A solution of the *o*-phenylenediamine (1 mmol) in absolute ethanol (10-15 mL) was slowly added to a solution of the aldehyde derivatives (1 mmol) in absolute ethanol and reflux the reaction mixture for 6-8 hrs. For synthesis of **SH 1**, **SH 2** and **SH 3** compound 4-nitrobenzaldehyde, 4-chlorobenzaldehyde and 4-dimethylbenzaldehyde were taken respectively. After completion of reaction, the contents were cooled and precipitates were formed which collected by filtration, then washed several times with cold ethanol (Moorthy, Vittal et al. 2017).



SH 1 = R = NO_2

SH 2 = R = Cl

SH 3 = R = $N(CH_3)_2$

Scheme 1: Synthesis of compounds (SH 1–3)

Pharmacological activities

Antioxidant activity

The antioxidant activity of synthesized compounds were determined by the ABTS free radical scavenging method.

ABTS scavenging activity

The antioxidant activity of synthesized compounds was measured by making its stock solutions in methanol (1000 µg/ml). For preparing stock solutions, 10 mg of each compounds dissolved in 10 ml of methanol. The prepared stock solutions were then diluted to different concentrations of 62.5, 125, 250, 500 and 1000 µg/ml. Same concentration was used for standard tocopherol. The radical was generated by reaction of 7 mM/L 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) in water with 2.4 mM/L potassium persulphate in equal quantities. The mixture was held in dark at 27 Ċ for 12 to 16 h. After incubation, the solution was further diluted i.e. 1 mL of the ABTS solution was diluted with 60 ml of methanol. For the assay, 5 ml of different concentrations of the tested synthesized compounds were allowed to react with 2 ml of ABTS solution for 7 min and Spectrophotometer was used to measured the absorbance at 734 nm. Blank solution was prepared by dissolving ABTS in methanol. The antioxidant activity of tested synthesized compounds was calculated by the following formula (El-Maati, Mahgoub et al. 2016).

% inhibition of ABTS activity = A-B/A × 100

A represents absorbance of blank and B represents absorbance of test sample.

Acute toxicity test

In vivo acute toxicity was evaluated for synthesized Schiff bases to determined toxic dose and optimum therapeutic dose using standard reported protocols with slight modifications. The mice were fasted for 12 h prior to the experiment. Doses of various concentrations (250, 500, 1000, 1500 and 2000 mg/kg) were prepared in tween 80 and normal saline. The mice were divided into control and three test groups each containing six animals. Each group was treated with

increasing dose of the synthesized compounds with predefined doses. Normal saline (10 ml/kg) was given to group 1 served as control group. Mice had access to food and water. All animals were observed for their initial effects i.e. toxicological, behavioral and allergic symptoms in first 6 hours and then mortality were noted for the next 24 h or till death. Histopathological examination of vital organ and biomarkers analysis of blood was attained (Rayaji and Agadihiremath 2016).

Elevated plus maze test

Elevated plus maze test was used to study exploratory behaviors of the animals. Anxiolytic potentials of synthesized Schiff bases were evaluated against standard dose of diazepam. All mice were divided into control, standard and three tests groups each containing six animals. The control group was treated with normal saline (10 ml/kg), the standard group was treated with diazepam (0.5 mg/kg, i.p) and three test groups were treated orally with synthesized compounds at a dose of 50 mg/kg body weight. Each mouse was placed at front of the open arm in centre of the maze. A video camera was used to record number of entries and time spent in open and closed arms for the next five min. The test was performed according to the described method with slight modifications (Mesfin, Asres et al. 2014). Anti-anxiety activity of the synthesized compounds were measured in animals such as the percentage of time spent in open arm was calculated as % = Number of seconds spent in open arms /300 total seconds × 100.

Light dark box test

Light dark box test was used to study exploratory behaviors of the animals. Anxiolytic potentials of synthesized Schiff bases were evaluated against standard dose of diazepam. All mice were divided into control, standard and three tests groups each containing six animals. The control group was treated with normal saline (10 ml/kg), the standard group was treated with diazepam (0.5 mg/kg, i.p) and three test groups were treated orally with synthesized compounds at a dose of 50 mg/kg body weight. Each mouse was placed at front of the hole in light box. A video camera was used to record number of transitions and time spent in light and dark box for the next five min. The test was performed according to the described method with slight modifications (Doukkali, Taghzouti et al. 2015). Anti-anxiety activity of the synthesized compounds were measured in animals such as the percentage of time spent in light compartment was calculated as % = Number of seconds spent in light compartment/300 total seconds × 100.

Hole board test

Hole board test was used to study exploratory behaviors of the animals. Anxiolytic and sedative potentials of synthesized Schiff bases were evaluated against standard dose of diazepam. All mice were divided into control, standard and three tests groups each containing six animals. The control group was treated with normal saline (10 ml/kg), the standard group was treated with diazepam (0.5 mg/kg, i.p) and three test groups were treated orally with synthesized compounds at a dose of 50 mg/kg body weight. Each mouse was placed in the center of the hole board. A video camera was used to record numbers of head dipping, hole crossing and rearing for the next five min. An anxiolytic effect was indicated by an increase in numbers of head dipping, hole crossing and rearing, while sedative effect was considered in a decrease of the above noted parameters as compared to the control group. The test was performed according to the described method with slight modifications (Cassani, Araujo et al. 2013).

Thiopental induced sleeping time

Plexiglass apparatus was used in sleeping model. All mice were divided into control, standard and three tests groups each containing six animals. The control group was treated with normal saline (10 ml/kg), the standard group was treated with diazepam (3

mg/kg, i.p) and three test groups were treated orally with synthesized compounds at a dose of 50 mg/kg body weight. Thiopental sodium was administered to each animal i.p at a dose of 60 mg/kg body weight, thirty minutes after the treated compound dose and 15 minutes after diazepam treatment. A video camera was used to record sleep latency and sleeping time i.e. the animals were observed for the time to lose their righting reflex (latent period) and duration of sleep (time between the loss and recovery of reflex) abruptly after thiopental sodium injection. The test was performed according to the described method with slight modifications (Aziz and Khan 2013).

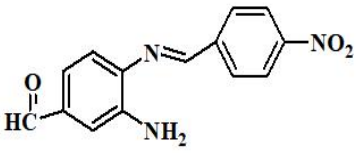
Statistical analysis

Means and standard error means (SEMs) for the data were calculated by using Excel, then all the statistical analysis were determined by using one way ANOVA followed by Dunnet's Multiple Comparison Test in GraphPad Prism.

RESULTS AND DISCUSSION

Synthesis

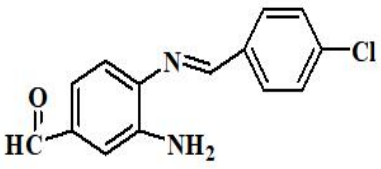
The synthesized benzaldehyde derived Schiff bases were prepared by adding *o*-phenylenediamine to their respective aldehydes solution which gave rise to three benzaldehyde derived Schiff bases. The synthesized compounds are shown in figure 1 – 3.



(*E*)-4-(4-nitrobenzylideneamino)-3-aminobenzaldehyde

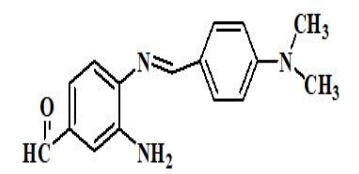
Figure 1: Compound SH 1

Analytical details



(*E*)-4-(4-chlorobenzylideneamino)-3-aminobenzaldehyde

Figure 2: Compound SH 2



(*E*)-4-(4-(dimethylamino) benzylideneamino)-3-aminobenzaldehyde

Figure 3: Compound SH 3

The structural elucidations of the compounds were based on their physical data as given below.

Table 1: Physical data of synthesized Schiff bases (SH 1–3).

Name	Mol For	Mol. Wt	App	Yeild	R.f	m.p.
SH 1	$C_{13}H_{11}N_3O_2$	241.25	Brown color powder	58.3%	0.63	299-301 ºC
SH 2	$C_{13}H_{11}ClN_2$	230.06	Orange color powder	67.1%	0.71	282-284 ºC
SH 3	$C_{15}H_{17}N_3$	239.14	Yellow color powder	75.6%	0.68	131-133 ºC

Characterization of Schiff bases

The synthesized compounds were characterized by their respective spectral data as mentioned below.

Compound SH 1:
IR (KBr) cm-1: 3459, 3368 (NH_2), 1583 (C=N); ^1H NMR (300 MHz, $CDCl_3$) ppm: 8.64 (s, 1H), 8.32 (d, 2H), 8.06 (d, 2H), 7.15-7.11 (m, 2H), 6.82-6.74 (m, 2H), 4.39 (br. s, 2H); Anal. calcd: C 64.72, H 4.60, N 17.42. Found: C 64.59, H 4.71, N 17.49.

Compound SH 2:
IR (KBr) cm-1: 3461, 3371 (NH_2), 1581 (C=N); ^1H NMR (300 MHz, $CDCl_3$) ppm: 8.53 (s, 1H), 7.81 (d, 2H), 7.49 (d, 2H), 7.21 (d, 2H), 6.81 (t, 2H), 4.33 (br. s, 2H); Anal. calcd: C 67.68, H 4.81, N 12.14. Found: C 68.09, H 4.69, N 11.90.

Compound SH 3:
IR (KBr) cm-1: 3463, 3369 (NH_2), 1589 (C=N); ^1H NMR (300 MHz, $CDCl_3$) ppm: 8.55 (s, 1H), 7.81 (d, 2H), 7.16-6.88 (m, 2H), 6.81 (d, 2H), 6.77 (d, 2H), 4.21 (br. s, 2H, NH_2), 2.91 (s, 6H, CH_3); Anal. calcd: C 75.28, H 7.16, N 17.56. Found: C 75.59, H 7.25, N 17.49.

Pharmacological activities

Antioxidant activity

The synthesized Schiff bases **SH 1**, **SH 2** and **SH 3** were screened for their possible antioxidant activity in ABTS free radicals scavenging method. In ABTS activity tocopherol was used as a positive control. The IC_{50} values for each compound were calculated. The results reveals that the compounds **SH 3**, **SH 2** and **SH 1** have good antioxidant activity respectively when compared to their respective taken control. However, the compound **SH 3** showed comparatively best IC_{50} value.

Table 2: ABTS scavenging activity of synthesized Schiff bases.

Test Sample	Sample concentration (µg/ml)	% Activity shown	IC_{50} (µg/ml)
SH 1	1000 500 250 125 62.5	$59.29 \pm 2.23^{*}$ $51.87 \pm 1.12^{*}$ 45.76 ± 1.33^{ns} $39.56 \pm 1.24^{*}$ $32.41 \pm 3.25^{**}$	567.6
SH 2	1000 500 250 125 62.5	61.87 ± 3.68^{ns} 55.69 ± 4.77^{ns} 49.42 ± 5.63^{ns} 43.27 ± 1.44^{ns} $34.51 \pm 1.26^{*}$	436.4
SH 3	1000 500 250 125 62.5	61.91 ± 4.16^{ns} 55.22 ± 3.17^{ns} 51.54 ± 2.29^{ns} 44.31 ± 2.48^{ns} $34.48 \pm 3.59^{*}$	415.833
Control (Tocopherol)	1000 500 250 125 62.5	71.18 ± 2.15 64.03 ± 3.14 56.12 ± 3.23 51.26 ± 2.32 47.31 ± 1.61	63.75

All values expressed as mean ± S.E.M (standard error mean) where n=6, ns= not significant, $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ when compared with the control by using two

way ANOVA followed by Bonferroni post test.

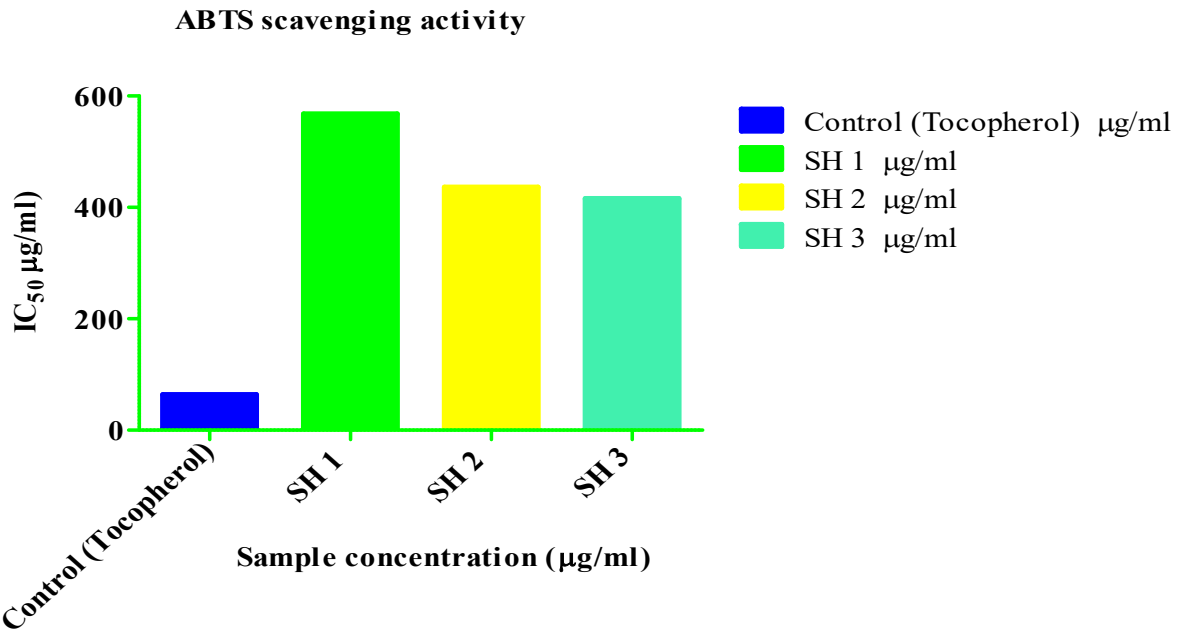


Figure 4: ABTS free radicals scavenging activity of synthesized Schiff bases against IC_{50}.

Acute toxicity test

In vivo acute toxicity was evaluated for synthesized Schiff bases. The mice were divided into control and three test groups each containing six animals. The animals were treated with increasing dose of the synthesized compounds using various concentrations (250, 500, 1000, 1500 and 2000 mg/kg). The control group treated with normal saline (10 ml/kg) produced no effects. The compound **SH 1** at doses of 250 and 500 mg/kg exhibited no mortality and the doses were considered as safe. However, at dose of 1000 mg/kg the toxic effects observed and percent mortality was 33.333%. When the dose increased to 1500 mg/kg the toxicity enhanced and mortality percentage was 50%. At dose of 2000 mg/kg the highest toxicity was observed and mortality percentage was 66.67%. The compound **SH 2** at doses of 250 and 500 mg/kg exhibited no mortality and the doses were considered as safe. However, at dose of 1000 mg/kg the percent mortality was 16.67%. When the dose increased to 1500 mg/kg the toxic effects observed and mortality percentage was 33.333%. Further increase in dose upto 2000 mg/kg the toxicity enhanced and mortality percentage was 50%. The compound **SH 3** at doses of 250, 500 and 1000 mg/kg exhibited no mortality and the doses were considered as safe. However, at dose of 1500 mg/kg the percent mortality was 16.67%. When the dose increased to 2000 mg/kg the toxic effects observed and mortality percentage was 33.333%.

Table 3: Acute toxicity effects of synthesized Schiff bases in mice.

Treatment	Dose (mg/kg)	Mortality after 24 hrs	Mortality (%)
Control	Normal saline (10 ml/kg)	–	–

SH 1	250 500 1000 1500 2000	0/6 0/6 2/6 3/6 4/6	0 0 33.333 50.00 66.67
SH 2	250 500 1000 1500 2000	0/6 0/6 1/6 2/6 3/6	0 0 16.67 33.333 50.00
SH 3	250 500 1000 1500 2000	0/6 0/6 0/6 1/6 2/6	0 0 0 16.67 33.333

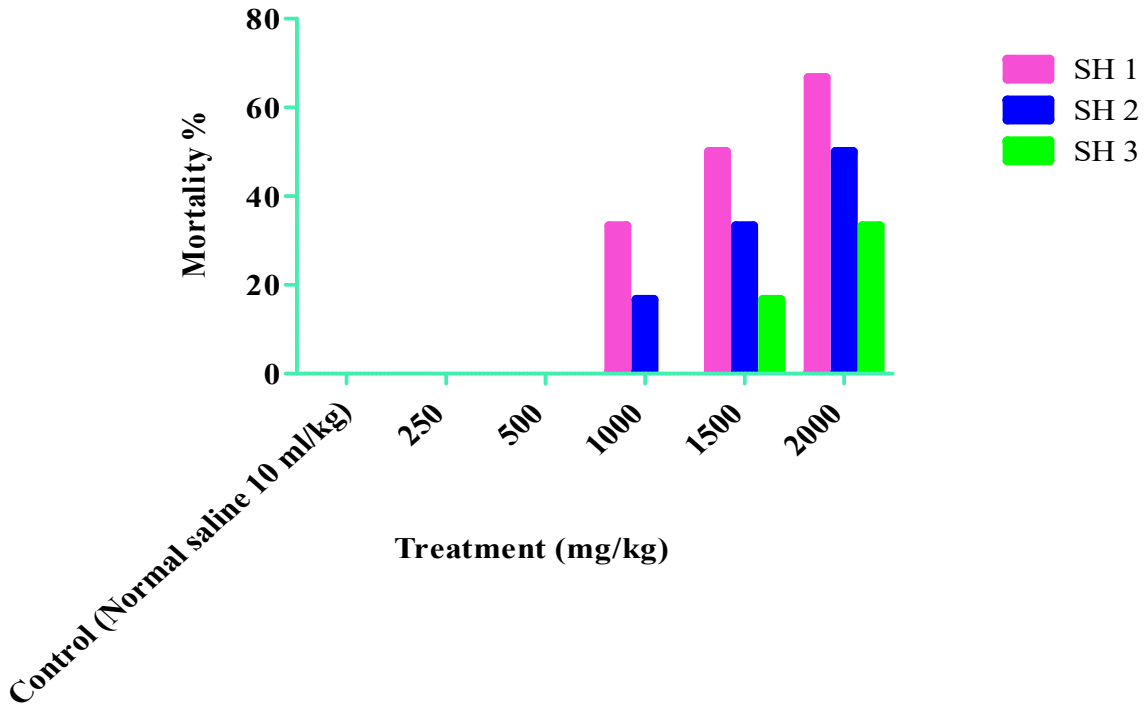


Figure 5: The effects of synthesized Schiff bases on percentage mortality in acute toxicity test.

Elevated plus maze test

Anxiolytic potentials of synthesized Schiff bases were evaluated against standard dose of diazepam. All mice were divided into control, standard and three tests groups each containing six animals. The control group was treated with normal saline (10 ml/kg), the standard group was treated with diazepam (0.5 mg/kg, i.p) and three test groups were treated orally with synthesized compounds at a dose of 50 mg/kg body weight. The compounds **SH 3**, **SH 2** and **SH 1** showed significant increase in percentage of time spent in open arm and number of open arm entries respectively when compared to the

control group, whereas significant reduction showed by these tested compounds respectively in percentage of time spent in closed arm as compared to control group. However, diazepam significantly increased the percentage of time spent in open arm and number of open arm entries.

Table 4: Effects of synthesized Schiff bases on elevated plus maze test in mice.

Treatment	Dose (mg/kg)	Time spent in open arm (sec/5min)	% of time(sec) spent in open arm	% of time(sec) spent in closed arm	Number of open arm entries	Number of closed arm entries
Control	Normal saline (10 ml/kg)	46.06 ± 5.274	15.353 ± 1.758	46.537 ± 1.327	9.88 ± 1.33	13.42 ± 1.762
Diazepam	0.5	118.67 ± 4.847***	39.557 ± 1.616***	24.333 ± 1.073***	12.682 ± 1.861ns	10.29 ± 1.240ns
SH 1	50	57.833 ± 2.714ns	19.278 ± 0.904ns	39.395 ± 0.582***	11.324 ± 1.018ns	12.572 ± 1.66ns
SH 2	50	74.34 ± 2.273***	24.78 ± 0.758***	34.884 ± 1.064***	11.83 ± 1.271ns	12.066 ± 1.08ns
SH 3	50	93.167 ± 3.63***	31.056 ± 1.21***	29.64 ± 0.841***	12.164 ± 1.537ns	11.744 ± 1.414ns

All values expressed as mean ± S.E.M (standard error mean) where n=6, ns= not significant, *p<0.05, **p<0.01 and ***p<0.001 when compared with the control by using one way ANOVA followed by Dunnet's Multiple Comparison Test.

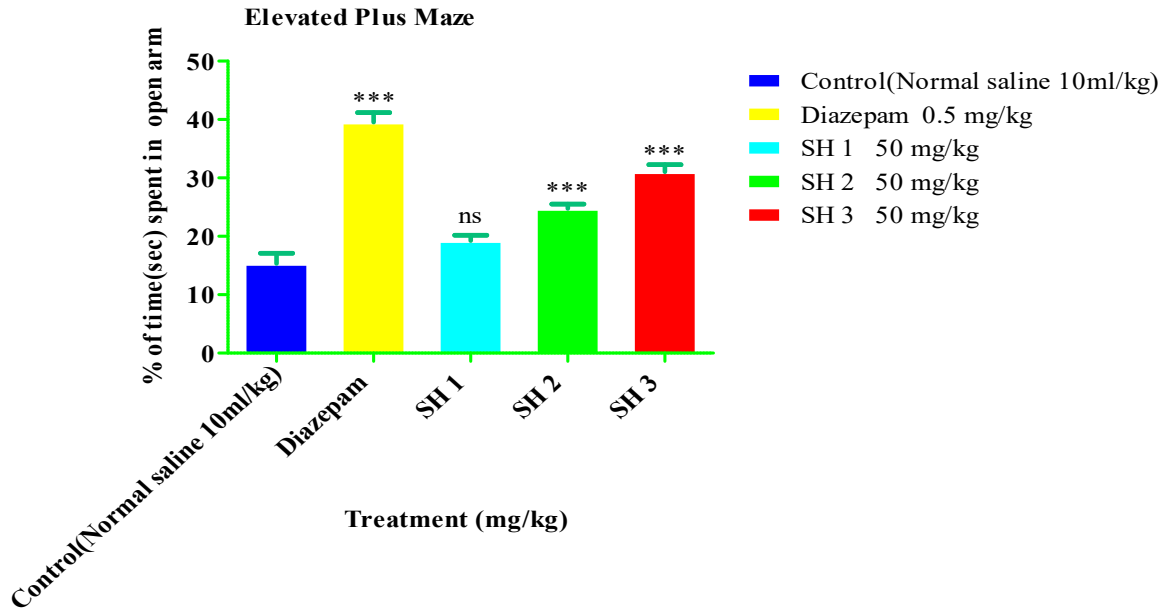


Figure 6: The effects of synthesized Schiff bases on percentage of time spent in open arm. Bars represent mean ± S.E.M from six mice.

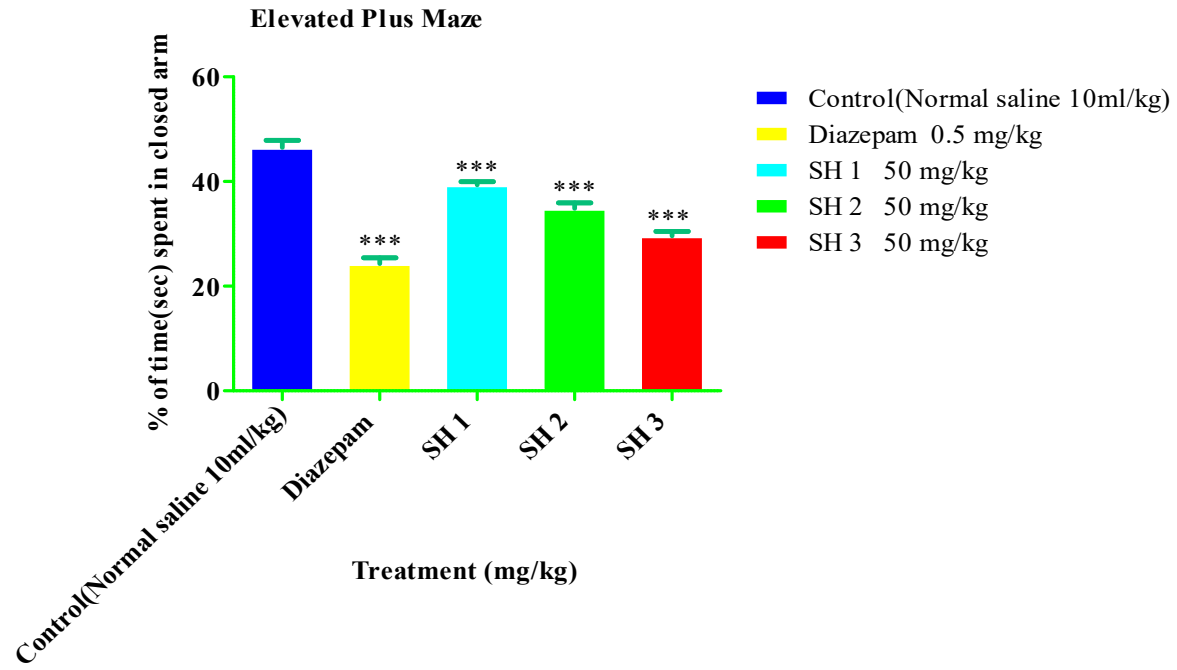


Figure 7: The effects of synthesized Schiff bases on percentage of time spent in closed arm. Bars represent mean ± S.E.M from six mice.

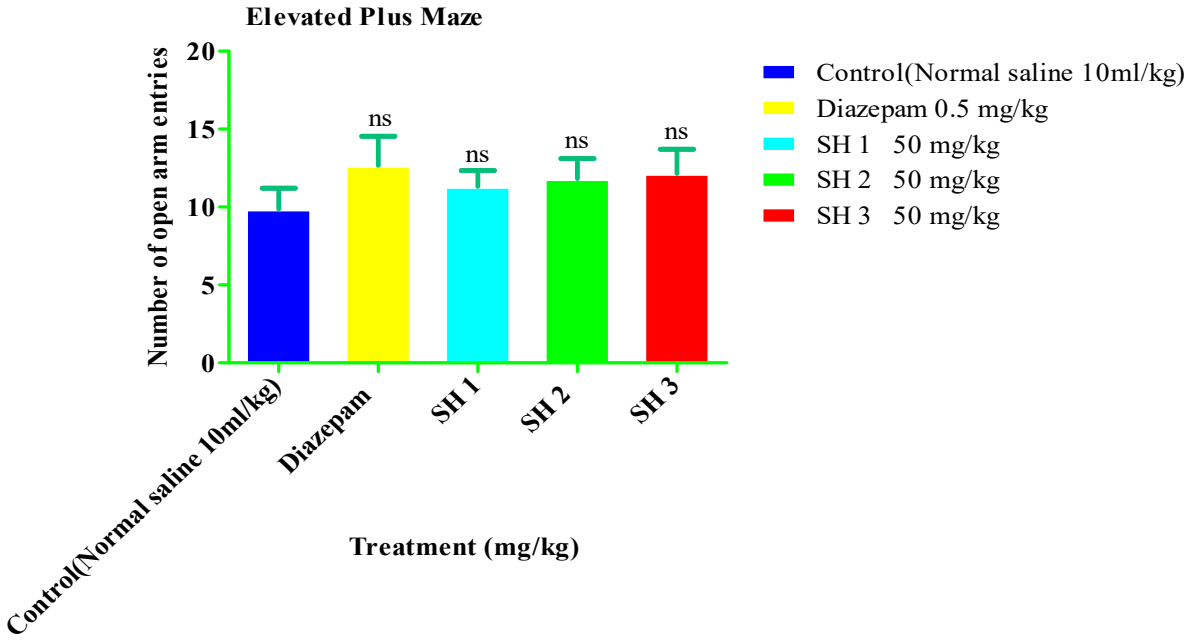


Figure 8: The effects of synthesized Schiff bases on number of open arm entries. Bars represent mean ± S.E.M from six mice.

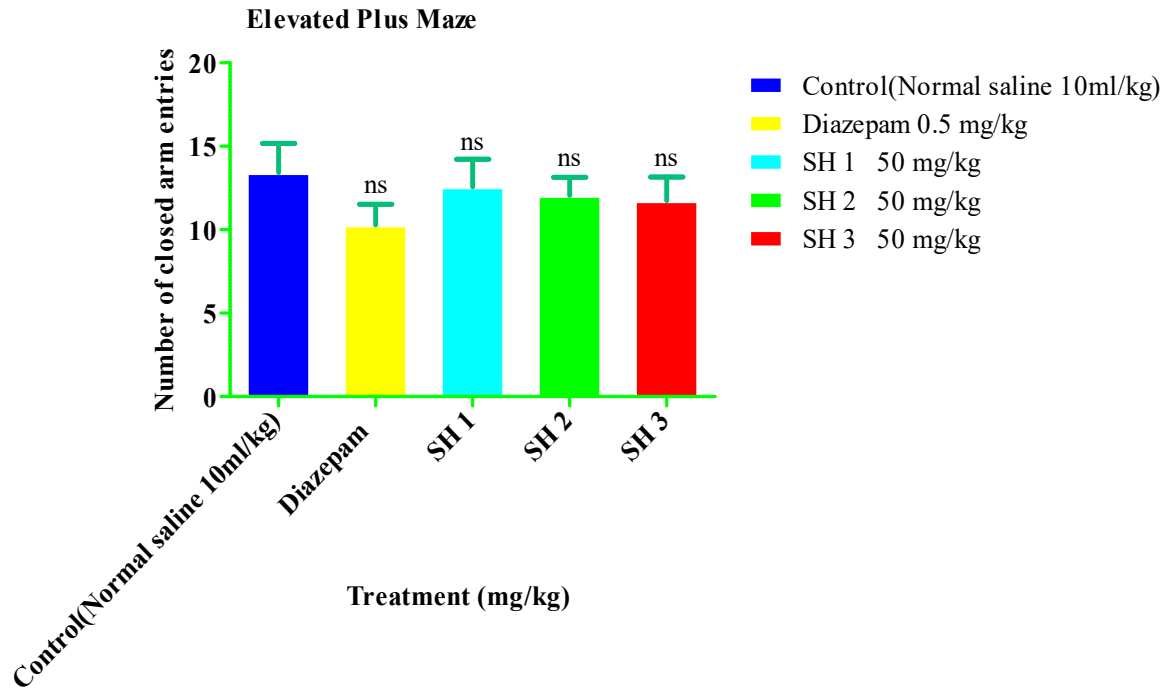


Figure 9: The effects of synthesized Schiff bases on number of closed arm entries. Bars represent mean ± S.E.M from six mice.

Light dark box test

Anxiolytic potentials of synthesized Schiff bases were evaluated against standard dose of diazepam. All mice were divided into control, standard and three tests groups each containing six animals. The control group was treated with normal saline (10 ml/kg), the standard group was treated with diazepam (0.5 mg/kg, i.p) and three test groups were treated orally with synthesized compounds at a dose of 50 mg/kg body weight. The compounds **SH 3**, **SH 2** and **SH 1** showed significant increase in percentage of time spent in light box and number of transitions respectively when compared to the control group, whereas significant reduction showed by these tested compounds respectively in percentage of time spent in dark box as compared to the control group. However, diazepam significantly increased the percentage of time spent in light box and number of transitions.

Table 5: Effects of synthesized Schiff bases on light dark box test in mice.

Treatment	Dose (mg/kg)	Time spent in light box (sec/5min)	% of time(sec) spent in light box	% of time(sec) spent in dark box	Number of transitions
Control	Normal saline (10 ml/kg)	88.64 ± 4.828	29.547 ± 1.609	86.673 ± 1.864	19.06 ± 1.910
Diazepam	0.5	173.84 ± 3.74***	57.947 ± 1.247***	45.15 ± 1.460***	26.284 ± 2.636*
SH 1	50	106.73 ± 1.916**	35.577 ± 0.639**	72.52 ± 1.242***	21.866 ± 1.854ns
SH 2	50	128.4 ± 2.73***	42.8 ± 0.91***	63.833 ± 0.84***	23.442 ± 0.822ns

SH 3	50	147.36 ± 3.037***	49.12 ± 1.012***	55.333 ± 0.252***	24.65 ± 0.240ns

All values expressed as mean ± S.E.M (standard error mean) where n=6, ns= not significant, *p<0.05, **p<0.01 and ***p<0.001 when compared with the control by using one way ANOVA followed by Dunnet's Multiple Comparison Test.

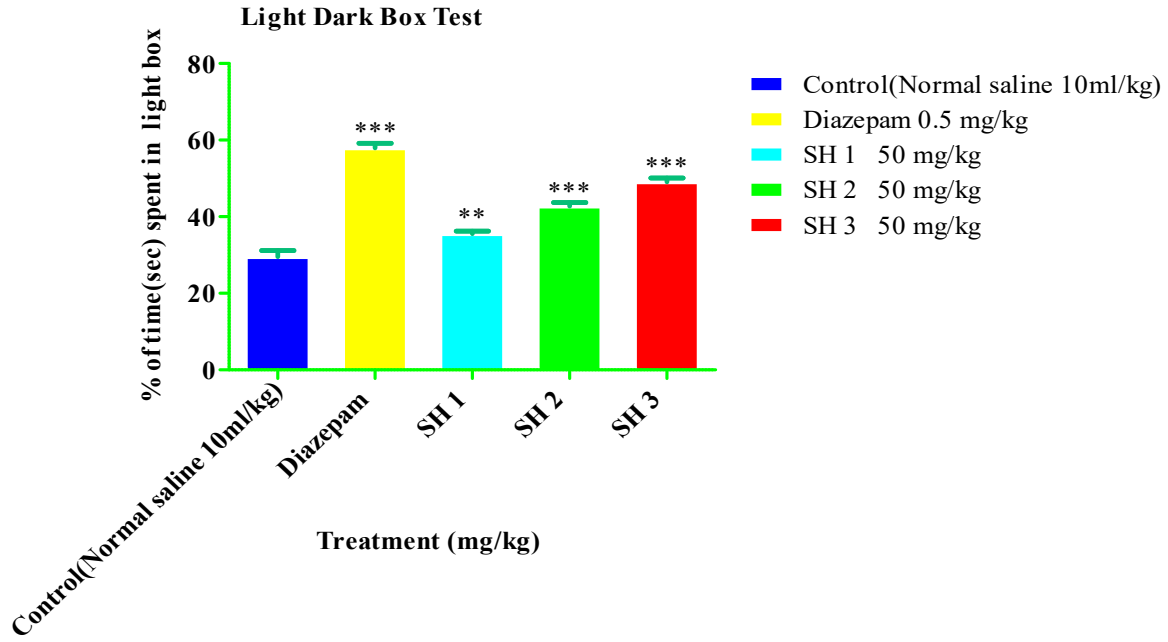


Figure 10: The effects of synthesized Schiff bases on percentage of time spent in light box. Bars represent mean ± S.E.M from six mice.

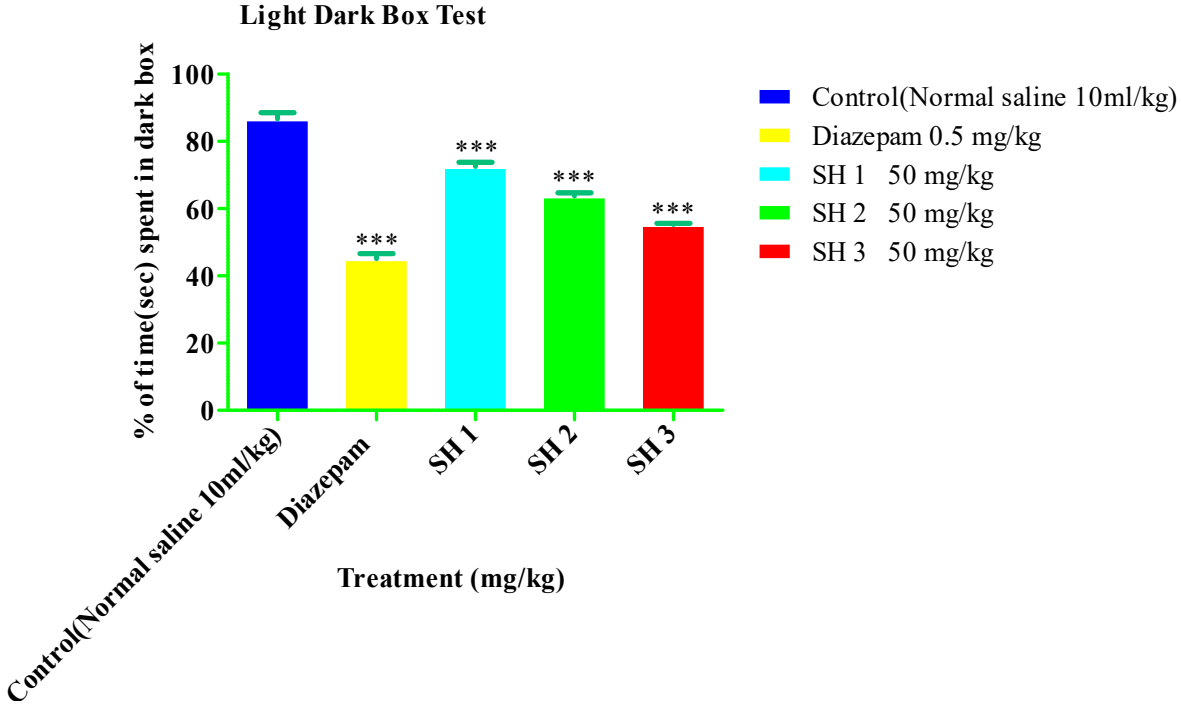


Figure 11: The effects of synthesized Schiff bases on percentage of time spent in dark box. Bars represent mean ± S.E.M from six mice.

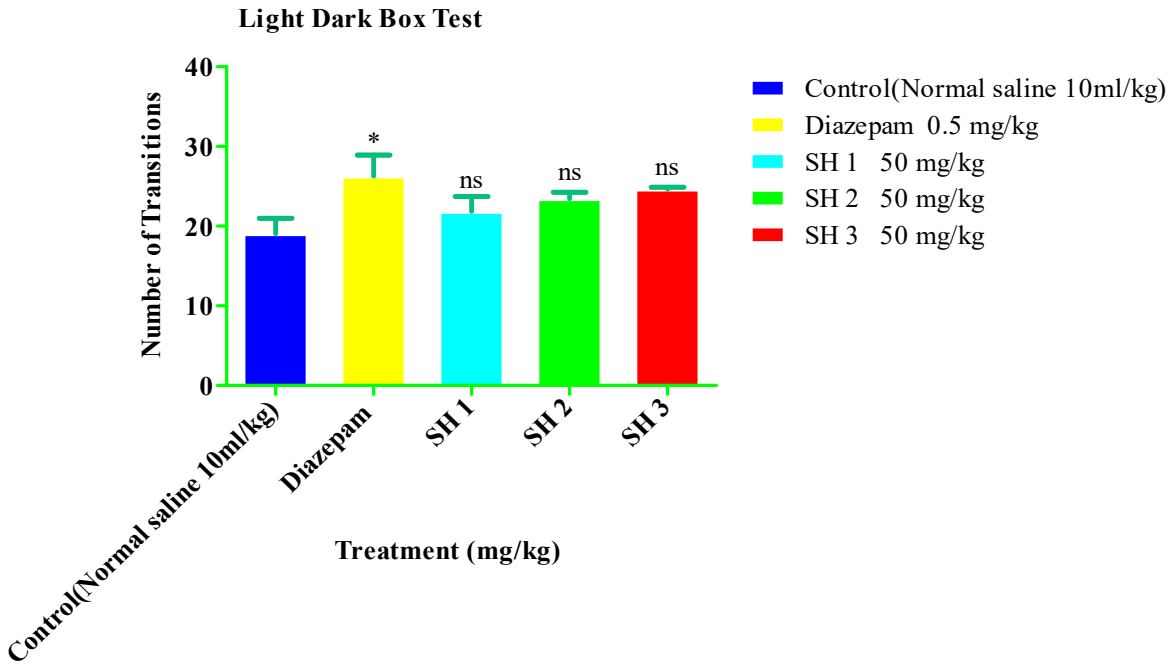


Figure 12: The effects of synthesized Schiff bases on number of transitions. Bars represent mean ± S.E.M from six mice.

Hole board test

Anxiolytic and sedative potentials of synthesized Schiff bases were evaluated against standard dose of diazepam. All mice were divided into control, standard and three tests groups each containing six animals. The control group was treated with normal saline (10 ml/kg), the standard group was treated with diazepam (0.5 mg/kg, i.p) and three test groups were treated orally with synthesized compounds at a dose of 50 mg/kg body weight. The compounds **SH 3**, **SH 2** and **SH 1** showed prominent increase in numbers of head dipping, hole crossing and rearing respectively as compared to the control group. Values exhibited no any sedative potentials with this treated dose of tested compounds. However, diazepam significantly increased the numbers of head dipping, hole crossing and rearing.

Table 6: Effects of synthesized Schiff bases on hole board test in mice.

Treatment	**Dose (mg/kg)**	**Number of head dipping**	**Number of hole crossing**	**Number of rearing**
Control	Normal saline (10 ml/kg)	28.12 ± 3.333	56.7 ± 2.116	11.37 ± 1.670
Diazepam	0.5	47.27 ± 1.55***	98.036 ± 4.44***	16.25 ± 1.48*
SH 1	50	32.64 ± 2.460ns	64.833 ± 1.927ns	12.14 ± 1.494ns

SH 2	50	36.17 ± 3.026^{ns}	$77.64 \pm 2.160^{***}$	13.836 ± 0.466^{ns}
SH 3	50	$41.065 \pm 2.184^{**}$	$85.7 \pm 3.96^{***}$	14.17 ± 0.960^{ns}

All values expressed as mean ± S.E.M (standard error mean) where n=6, ns= not significant, $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ when compared with the control by using one way ANOVA followed by Dunnet's Multiple Comparison Test.

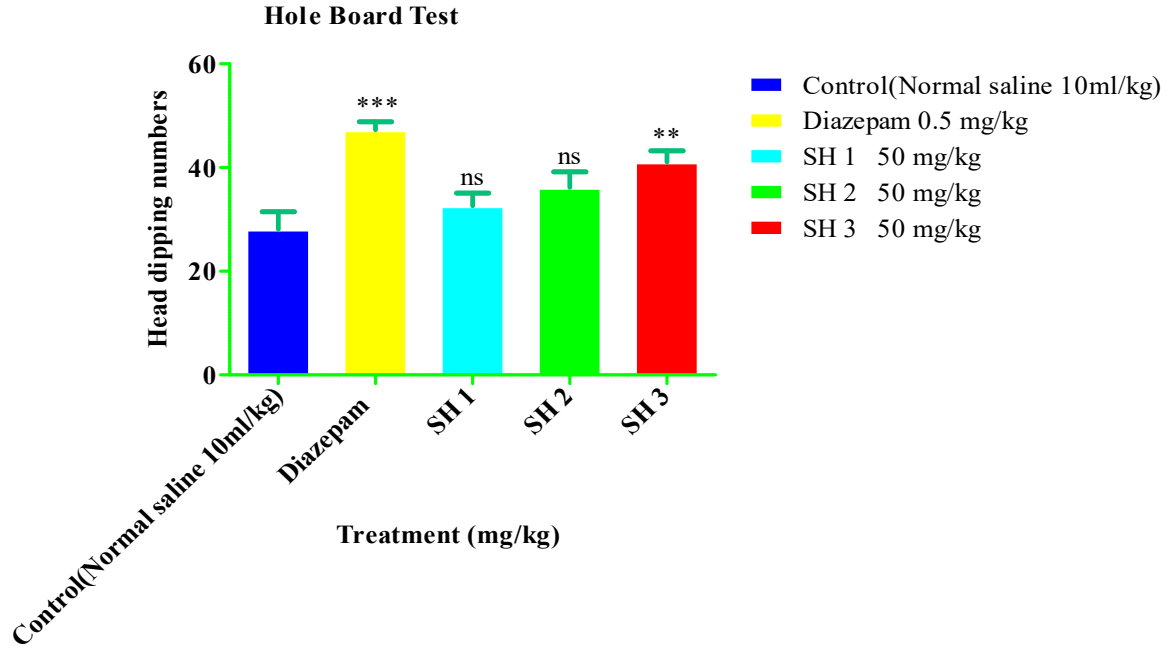


Figure 13: The effects of synthesized Schiff bases on numbers of head dipping. Bars represent mean ± S.E.M from six mice.

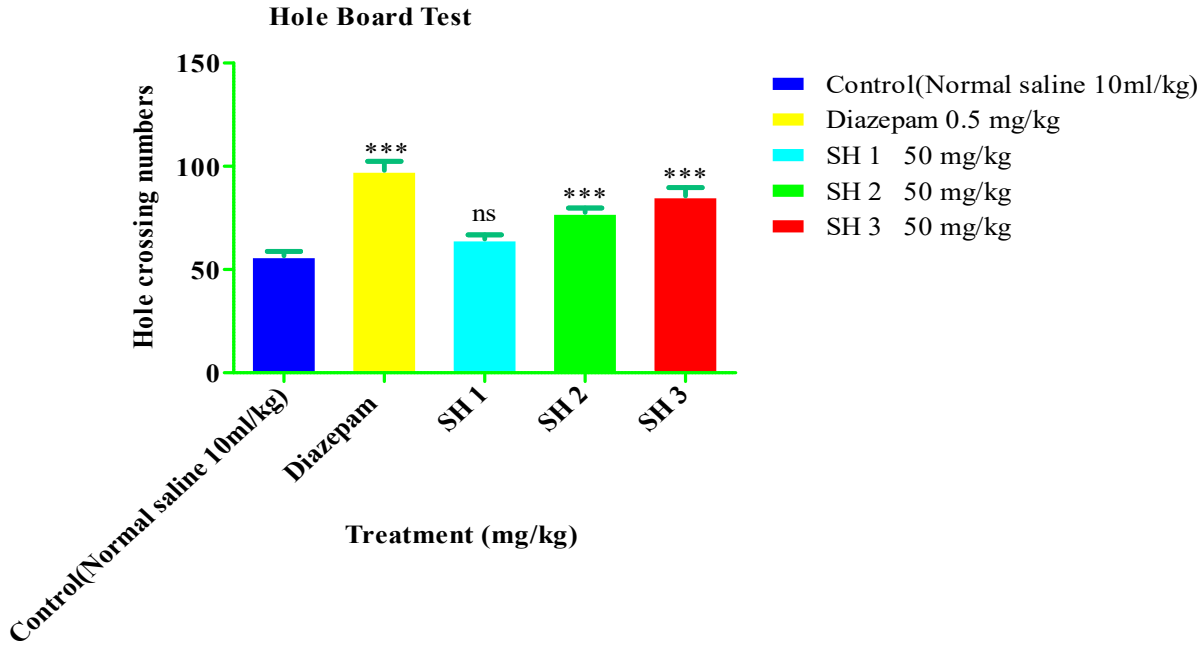


Figure 14: The effects of synthesized Schiff bases on numbers of hole crossing. Bars represent mean ± S.E.M from six mice.

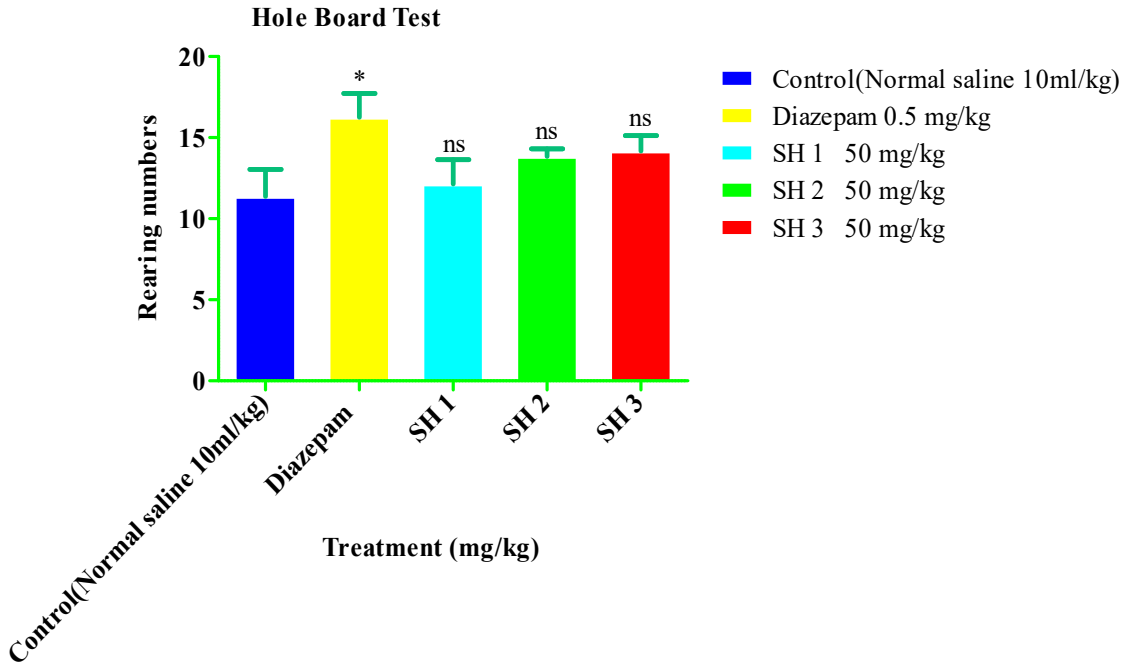


Figure 15: The effects of synthesized Schiff bases on numbers of rearing. Bars represent mean ± S.E.M from six mice.

Thiopental induced sleeping time

All mice were divided into control, standard and three tests groups each containing six animals. The control group was treated with normal saline (10 ml/kg), the standard group was treated with diazepam (3 mg/kg, i.p) and three test groups were treated orally with synthesized compounds at a dose of 50 mg/kg body weight. Thiopental sodium was administered to each animal i.p at a dose of 60 mg/kg body weight, thirty minutes after the treated compound dose and 15 minutes after diazepam treatment. The compounds **SH 3**, **SH 2** and **SH 1** showed prominent reduction in sleep latency respectively as compared to control group, whereas prominent increase showed by these tested compounds respectively in sleeping time (hypnosis) as compared to the control group. However, diazepam significantly prolonged the sleeping time and reduced sleep latency.

Table 7: Effects of synthesized Schiff bases on thiopental induced sleeping time in mice.

All values expressed as mean ± S.E.M (standard error mean) where n=6, ns= not

Treatment	Dose (mg/kg)	Sleep latency (min)	Sleeping time (min)
Control (Thiopental +NS)	60	8.26 ± 0.447	38.584 ± 2.686
Thiopental + Diazepam	3	6.020 ± 0.146*	75.166 ± 2.844***
Thiopental + SH 1	50	7.882 ± 0.582^{ns}	43.62 ± 1.042^{ns}
Thiopental + SH 2	50	7.422 ± 0.760^{ns}	46.864 ± 0.640^{ns}
Thiopental + SH 3	50	6.834 ± 0.347^{ns}	50.68 ± 3.024**

significant, *p<0.05, **p<0.01 and ***p<0.001 when compared with the control by using one way ANOVA followed by Dunnet's Multiple Comparison Test.

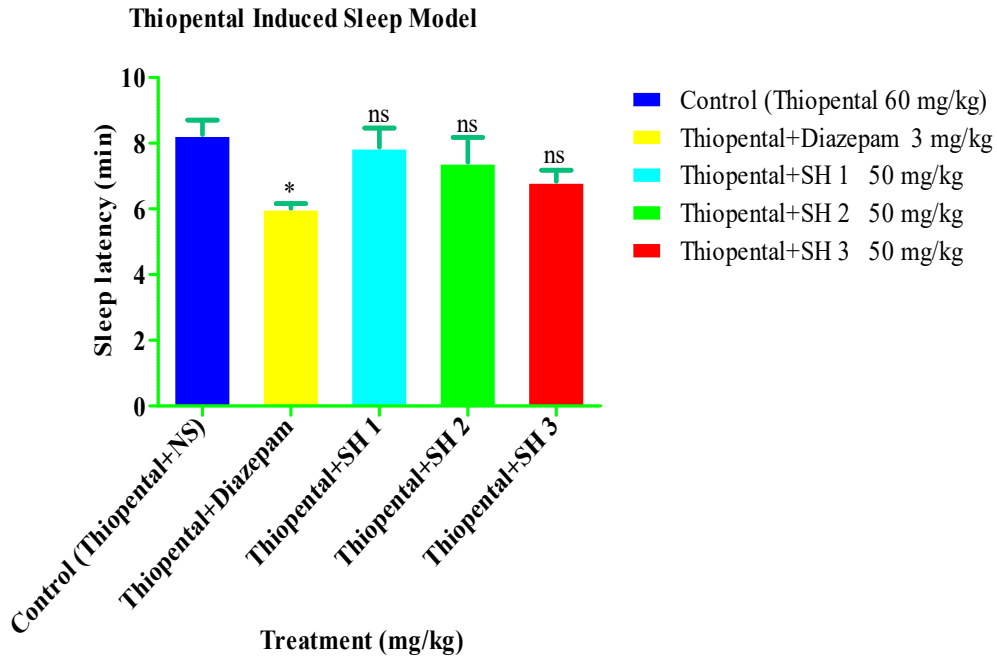


Figure 16: The effects of synthesized Schiff bases on sleep latency. Bars represent mean ± S.E.M from six mice.

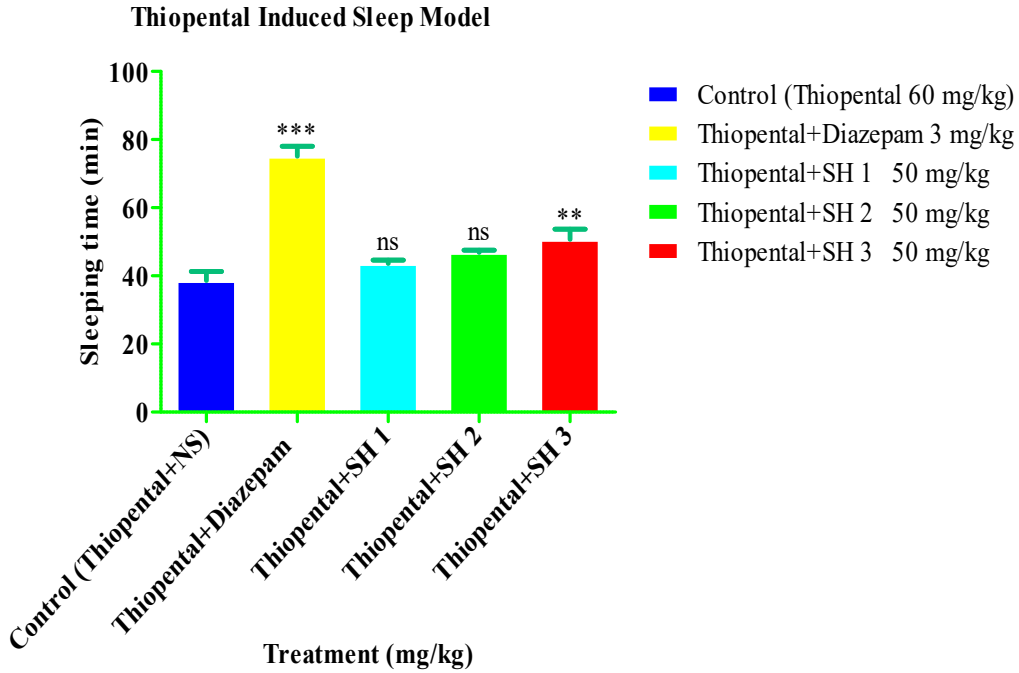


Figure 17: The effects of synthesized Schiff bases on sleeping time. Bars represent mean ± S.E.M from six mice.

DISCUSSION

Schiff bases have structural diversity and hence synthesized in large amount by condensation of primary amine and aldehyde through different methods, such as the presence or absence of a catalyst (acid or base) in an alcoholic solution, microwave assisted or solvent-free mechano-chemical synthesis (Griffiths, Dokorou et al. 2016). In our study the IC_{50} values for ABTS free radicals scavenging activity were calculated. Tocopherol used as a positive control in ABTS activity. Low value of IC_{50} indicates the greater antioxidant potential. The results demonstrated that the compounds **SH 3**, **SH 2** and **SH 1** have good antioxidant activity respectively when compared with the control. However, the compound **SH 3** showed comparatively best IC_{50} value **(Table 2)**. The acute toxicity study of synthesized compound **SH 1** shows that the compound is safe upto 500 mg/kg. The compound **SH 2** is safe upto 1 g/kg and the compound **SH 3** is safe upto 1.5 g/kg **(Table 3)**. Benzodiazepines have been used for many years as a standard treated drug for anxiety disorders. Anxiety is the alteration in behaviors of animals in new environment and mainly produced by the fluctuations occur in the neurotransmitters, GABA, serotonin, adrenaline and dopamine. The elevated plus maze is mostly use for the evaluation of anxiolytic effects. The anxiolytic effect was measured by the time spent in open and closed arms and the number of entries (Sheela, Srinivasan et al. 2017). Depression is the imbalances produced by the destruction of few chemicals or hormones in the brain with common indications including an inability to experience pleasure and interest, loss of concentration, social anxiety, self-doubt, sleep and appetite disorder etc. Synthetic indole alkaloids have broad medicinal applications (Hamid, Ramli et al. 2017). Muslim et al., (2017) synthesized oxazepine derivatives and reported for their antidepressant property (Jasim, Muslim et al. 2017). In our study the synthesized compounds **SH 3**, **SH 2** and **SH 1** showed a significant increased in the time spent and number of entries in open arms respectively and thus the results demonstrate the anxiolytic potential of the compounds **(Table 4)**. Diazepam mainly act on benzodiazepine receptors and produce standard anxiolytic effects. The light dark box test is usually use for evaluation of anxiolytic effects in animals models. It has been reported that animals spent more time in dark box as compared to light box (Aslam and Sultana 2016). In our study the synthesized compounds **SH 3**, **SH 2** and **SH 1** showed a significant increased in time spent in the light box and number of transitions respectively, the results reveal the anxiolytic potential of the compounds **(Table 5)**. To measure the response of animals in new environment the hole board test is usually carry out. In hole board test different behavioral parameters are comprises to measure the anxiety in animals such as emotionality change has confirmed by head dipping numbers with changing locomotion (Archana 2013). In our study the synthesized compounds **SH 3**, **SH 2** and **SH 1** showed a prominent increased in numbers of head dipping, hole crossing and rearing respectively, and the results illustrate the anxiolytic like effect of the synthesized compounds **(Table 6)**. To evaluate the sedative hypnotic effect of drugs the thiopental induced sleeping time test is perform. Thiopental sodium occur in barbiturates class of drugs hence induces sleep in humans and rodents. Thiopental sodium act on GABA receptor complex thus shows GABA mediated hyper polarization of post synaptic neurons. The CNS depressant action is executed by increasing the influx of chloride ions through chloride channels opening and blocking of the excitatory glutamate receptors that also enhanced GABA activity (Sultana, Mannan et al. 2018). In our study the synthesized compounds **SH 3**, **SH 2** and **SH 1** showed a prominent reduction in

sleep latency and prominent prolongation in sleeping time respectively **(Table 7)**. The results exhibit a relationship between the CNS depressant effect of synthesized compounds and diazepam.

Conclusion

The benzaldehyde derived Schiff bases **SH 1**, **SH 2** and **SH 3** were synthesized and screen for the antioxidant, acute toxicity, anxiolytic and sedative potentials. The compounds possess good antioxidant activity. In acute toxicity study the toxicity was dose dependent, the LD_{50} of compound **SH 1** and **SH 2** was 1500 and 2000 mg/kg and the compound **SH 3** was safe upto 1.5 g/kg. The anxiolytic activity was measured through different models and sedative potentials were examine in thiopental induced sleeping time test.

The compound **SH 1** was found to possess poor pharmacological effects while the compounds **SH 2** and **SH 3** showed significant pharmacological results which prompted us to study their other pharmacological activities like antiviral, antibacterial, anticancer and so on. Also, a relationship was showed between the CNS depressant effect of synthesized compounds and diazepam since further studies are require to exposed the underlying mechanism in detail.

This study will clear the way for researchers in future and further studies are require to exposed the underlying mechanism in detail.

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