Solanesol Mediated SIRT-1 Signaling Activation Prevents Neurobehavioral and Neurochemical Defects in Ouabain-induced Experimental Model of Bipolar Disorder in Rats

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Research Article

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Abstract

Bipolar disorder (BD) is a serious and widespread chronic mental condition characterized by mood swings ranging from depressive lows to manic highs. Several studies have linked SIRT-1 (silent mating type information regulation-2 homologs) signalling downregulation to the progression of BD and other neurological dysfunctions. The purpose of this study was to investigate the neuroprotective potential of solanesol(SNL) in rats with brain intoxication caused by intracerebroventricular (ICV) injections of ouabain(OUA), with a particular focus on its influence on the SIRT-1 signaling activator in the brain. The goal of this study was to investigate the neuroprotective potential of Solanesol (SNL) in rats treated with ICV-OUA injection, with a special emphasis on its effect on the SIRT-1 signalling activation in the brain. Ouabain (OUA) is a cardiac glycoside that inhibits the Na+/K+-ATPase (sodium-potassium adenosine triphosphatase). SNL is an active phytoconstituent belongs to the Solanaceae family, derived from the plant Nicotiana tabacum. SNL is employed as a precursor for the production of CoQ10 (Coenzyme Q10), which has potent antioxidant and neuroprotective properties. Lithium (Li), an important mood stabiliser drug employed as a control in the present study. This study looked at the neuroprotective potential of SNL at doses of 40 and 80 mg/kg in ICV-OUA injections, which caused BD-like neurobehavioral deficits in Wistar rats. Wistar rats were divided into eight groups (n=8) and given 1 mM/0.5 l OUA injections for three days. Long-term SNL and lithium administration can reduce the number of rearing and crossings and time spent in the centre, locomotive activity, and immobility time. According to the findings of this study, SNL increases the levels of SIRT-1 in CSF, blood plasma, and brain homogenate samples. In addition, SNL modulates the apoptotic markers like Caspase-3, Bax (pro-apoptotic), and Bcl-2 (anti-apoptotic) in rat brain homogenates and blood plasma samples. Mitochondrial-ETC complexes enzymes including complex-I, II, IV, V, and CoQ10 were also resorted after the long term administration of SNL. Furthermore, SNL reduced inflammatory cytokines (TNF-α, IL-1β) while restoring neurotransmitter (serotonin, dopamine, glutamate, and acetylcholine) levels and level of oxidative stress markers. Our neurochemical observations could be validated as diagnostic biomarkers in BD-like conditions. As a result, SNL as SIRT-1 signaling activator could be a promising therapeutic target for the treatment of BD.

1.0 Introduction

BD is a severe mental illness typified by depression, mania, psychosis, and neurocognitive deficits (Waddington et al., 2021; McCarthy et al., 2014). It is one of the ten most debilitating psychiatric disorders globally (Rhee et al., 2020). Based upon family and twin studies, a genetic basis of the illness is strongly suspected, but the genes responsible for BD remain primarily unknown (McGuffin et al., 2003). It is a heritable mental illness with complex etiology (Uher and Zwicker, 2017), linked to an increased risk of morbidity, mortality, and comorbidity in psychiatry (Serra et al., 2017; Mullins et al., 2021). BD affects individuals from a young age and is related to physical morbidity and early death (Walker et al., 2015).

BD is uncommon among medical diseases in that its manifestations alternate between two distinct n (Hirschfeld et al., 2014). These key characteristics are met

by the experimental animal model of mania induced by OUA, a Na+/K+-ATPase enzyme inhibitor, making it suitable for studying numerous behavioural and neurochemical aspects of BD (Jornada et al., 2011). OUA dose-dependently increases locomotor activity in rats, associated with manic-like behavior (Valvassori et al., 2015). The Na+/K+-ATPase is an ion transporter that influences neuronal excitability, electrochemical gradient, resting membrane potential, and neurotransmitter release and uptake, in addition to maintaining Na+/K + equilibrium (Ladol and Sharma et al., 2021). According to reports, the validity of animal models in psychiatric diseases should identify the three basic criteria: face validity, construct validity, and predictive validity (Nestler and Hyman, 2011). Face validity refers to a model's ability to reproduce the symptoms of a particular condition. On the other hand, construct validity relates to the model's ability to recreate some pathophysiological components of the disease. Finally, predictive validity examines if the medications used to treat a disorder can reverse the symptoms observed in the animal model (Valvassori et al., 2013). Furthermore, ICV injection of OUA into rats results in neurochemical changes similar to those reported in BD patients and abnormalities in neurotrophic factors, mitochondrial function and causes oxidative stress (Lopes-Borges et al., 2015).

SIRT-1 is a protein found in the adult brain and spinal cord, specifically the amygdala, hippocampus, cerebellum, hypothalamus, and deeper into the neuronal body (Schwartz et al., 2000; Michan and Sinclair., 2007). SIRT-1 is a transcription factor that regulates stress response, genome maintenance, axon elongation, dendritic branching, and endocrine activity (Li et al., 2013). SIRT-1 participates in several functions, including transcription, metabolism, genome maintenance, neural progenitor fates, axon elongation, dendritic branching, and endocrine function (Herskovits and Guarente, 2014; Donmez et al., 2013).

Deacetylation of this protein affects cellular processes like aging, inflammation, apoptosis, mitochondrial biogenesis, and stress resistance (Aguirre et al., 2015; Alcendor et al., 2007).

SIRT-1 deficiency results in hyperglycemia and osteoporosis (Bartoli-Leonard et al., 2019). SIRT-1 dysregulation promotes disease progression by increasing oxidative damage and inflammation (Elibol and Kilic et al., 2018). In a recent study, SIRT-1 overexpression was demonstrated to increase cell survival, decrease cell apoptosis, and reduce the release of pro-inflammatory cytokines (Li et al., 2018). SIRT-1 also influences metabolism and longevity by regulating the production of hypothalamic peptide hormones (Nillni et al., 2016). SIRT-1 specificity increases in essential metabolic pathways in hypothalamic circuits, linked to alterations in SIRT-1 downstream factors such as FoxO transcription factors (Baldo et al., 2018). In light of these facts, we reviewed recent studies concerning the relationship between increasing SIRT-1 protein levels rather than reducing SIRT-1 expression and regulating several disease-related states such as obesity, cardiovascular disease, and neurodegeneration. SIRT-1 deficiency affects transcription factors (p53, PGC-1, NF-B, and FOXO) as well as molecular changes such as gene expression, influencing brain plasticity, Th17 cell inhibition, and interleukin-1 production (Lee et al., 2016; Zhong et al., 2012).

SIRT-1 activation appears to have beneficial effects in BD (Alageel et al., 2018), MS (Zhao et al., 2015), Parkinson's disease (PD) (Feng et al., 2015), and Alzheimer's disease (AD) (Donmez et al., 2013). Recent

research has revealed a link between SIRT-1 downregulation and disease progression, as well as an increase in oxidative stress and inflammation (Singh et al., 2017). Patients with obesity experienced higher fatty acid oxidation due to a considerable decrease in SIRT-1 levels, which were linked to increased oxidative stress (Elibol and Kilic et al., 2018).

SIRT-1 downregulation has been linked to a depressive phase in humans (Zhou et al., 2020). According to Abe-Higuchi et al., continuous stress decreases SIRT-1 activity in the dentate gyrus, and pharmacological or genetic elimination of hippocampus SIRT-1 increases depression-like behaviours. Chronic stress may result in the formation of depression-related phenotypes and aberrant dendritic structures, which could be prevented by activating SIRT-1. In a mouse model of chronic stress-induced depression, researchers also investigated the involvement of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) as potential downstream targets of SIRT-1. It was observed that activating hippocampus SIRT-1 increased ERK1/2 phosphorylation under stressed conditions and that viral-modulated hippocampal ERK2 function was related to antidepressive and depressed behaviours (Abe-Higuchi et al., 2016). Another research reveals that chronic variable stress (CVS) increased depressive-like behaviour, which was linked to a decrease in ERK1/2 phosphorylation, Bcl-2 expression, and H4 (K12) acetylation in hippocampal subregions following chronic stress (Ferland et al., 2013). SIRT-1 deficiency enhanced dopamine neurotransmission, which is involved in developing bipolar disorder manic-likeee episodes (Zhu et al., 2019).

SNL is a solanaceous crop produced by the 'Nicotiana Tobacco' from the Solanaceae family.SNL is a long-chain polyisoprenoid alcohol molecule that contains nine isoprene units and is also recognized as a CoQ10 precursor (Rajdev et al., 2020). CoQ10 (2, 3 dimethoxy-5-methyl-6-decaprenyl benzoquinone) is the most prevalent coenzyme in human mitochondria, with ten repeating isoprene units. It is also known as ubiquinone. CoQ10 is a component of the electron transport chain that accepts an electron from complex-I and pumps protons across the inner mitochondrial membrane before passing the electrons to complex-II, responsible for the generation of ATP. It reduces oxidative damage to neurons and improves behavioural function in animals (Sharma et al., 2019).

SNL is used to treat ulcers and has a variety of pharmacological effects, including antibacterial, anti-inflammatory, and anti-tumor activities. In the pharmaceutical sector, it is used to produce coenzyme Q10, vitamin K2, and N-solanesyl-N, N'-bis(3,4-dimethoxybenzyl) ethylenediamine (SDB) (Yan et al., 2015).

Amyotrophic lateral sclerosis (ALS) (Alam et al., 2020) and multiple sclerosis (MS) are two other neurodegenerative disorders that potentially benefit from SNL treatment (Sharma et al., 2021). CoQ10 precursors have been shown to protect against migraine (Sandor et al., 2005) and Huntington's disease (Mehan et al., 2018). CoQ10 precursors have been shown to prevention against neurodegenerative diseases such as Parkinson's disease (Shults et al., 2004) and amyotrophic lateral sclerosis (ALS) (Matthews et al., 1998). It has also been effective in Alzheimer's disease, multiple sclerosis (DeLegge and Smoke, 2008), and bipolar disorder (BD) (Forester et al., 2015). In addition to its anti-oxidant and anti-aging properties, it is supposed to boost the body's immune system and improve cognitive function (Guo

et al., 2008). CoQ10 has also been demonstrated to protect against hepatic IR injury via modulating the SIRT-1 pathway (Mahmoud et al., 2019).

As a SIRT-1 signalling activator, SNL has been shown to have neuroprotective properties against Alzheimer's disease (Jaiswal et al., 2019), intracerebral haemorrhage (ICH) (Rajdev et al., 2020), and autism (Sharma et al., 2019). It also has neuroprotective benefits against MS (Sharma et al., 2021) and HD (Mehan et al., 2010).

Lithium (Li), an important mood stabilizer, effectively prevents such behavioural changes (Jornada et al., 2011).On the other hand, hypoactivity is insufficient to imitate a state of depression, and more research is required to support this hypothesis. The “Na+/K+-ATPase hypothesis,” which suggests that decreased enzyme activity has a crucial role in the onset of manic and depressed mood episodes in BD, was used to build the OUA model of mania (El-Mallakh and Wyatt, 1995). Several studies have found that the activity of the Na+/K+-ATPase is reduced in bipolar patients (Banerjee et al., 2012; El-Mallakh et al., 1983).

The mood-stabilizing therapeutic effects of lithium were just identified in the absence of any relevant mechanistic knowledge of BD (Can et al., 2014). Current medications, such as lithium alone or in combination, are successful in 60 percent of continuously treated individuals (Pisanu et al., 2018). Although olanzapine, quetiapine, and ziprasidone (Monti, 2016), as well as valproate, carbamazepine, and lamotrigine (Joas et al., 2017), are generally valuable for reversing manic episodes and preventing future events. However, they are of little, if any, use in the acute treatment of depressive episodes. Furthermore, conventional antidepressants, whether administered alone or in combination with mood stabilizers or antipsychotics, are generally ineffective for treating depressive episodes and may encourage mood flipping in a group of people with BD (McInerney and Kennedy, 2014).

In this investigation, we hypothesized that the SNL could upregulate SIRT-1 target signaling mechanisms and, as a result, can alleviate neuropathological changes in OUA-induced BD-like rats through its putative target-modulating properties. Furthermore, SNL could be employed in combination with other standard drug regimens to provide a promising pharmacological strategy for people suffering from neuropsychiatric illnesses such as BD. As a result of these promising pharmacological effects, we investigated and evaluated SNL's neuroprotective profile in rat brain homogenate, blood plasma, and CSF samples, with the goal of using these biological samples as effective and provable diagnostic biomarkers during the early stages of neurodegeneration and neuropsychiatric disorders.

2.0 Material And Methods

2.1 Experimental animals

Adult Wistar rats (220-250gm, nine weeks of age, either sex) were collected from the ISF College of Pharmacy Central Animal House in Moga, Punjab. These animals were evenly divided and housed in polyacrylic cages with a wire mesh top and soft bedding under typical husbandry circumstances of a 12-

and water, and a temperature of 23 ± 2°C. According to the

requirements of the Government of India, the experimental procedure was approved by the Institutional AnimalEthics Committee (IAEC) with a registration number.816/PO/ReBiBt/S/04/CPCSEA as protocol no. ISFCP/IAEC/CPCSEA/Meeting No: 28/2020/Protocol No.463. Animals were acclimatized to laboratory conditions before being used in experiments.

2.2 Drugs and chemicals

OUA was purchased from Sigma–Aldrich (USA). Ex-gratia samples of SNL from BAPEX (India) and Lithium carbonate from Sun Pharma were provided. All of the other chemicals employed in the experiment were of analytical grade. Before use, the medication and chemical solutions were freshly made. Oral administration of SNL dissolved in water (with 2% ethanol) (p.o.) (Mehan et al., 2017).

2.3 Experimental animal grouping

A total of 48 Wistar rats (either sex), nine weeks old, were employed during the course of the 28-day protocol schedule. These rats were kept in a polyacrylic cage with a wire mesh top and soft bedding (38 cm 32 cm 16 cm; 3–4 rats per cage) at a regulated temperature (22°C ± 2°C) and humidity (65–70 %) with artificial illumination (12 h/12 h light/dark cycle, lights on at 6:00 AM). Their bedding consisted of residue-free wood shavings that had been sanitized. These animals had unrestricted access to a standard chow diet as well as purified water. To avoid the effects of the circadian rhythm, the entire experimental protocol schedule was completed between 9:00 AM and 1:00 PM. They were randomly divided into eight groups (n = 6 per group). Group 1 vehicle control; Group 2 Sham control; Group 3 SNL perse (80mg/kg p.o.); Group 4 OUA (1 mM/0.5µl/5min/Unilateral/ICV injection); Group 5 OUA + SNL (40mg/kg, p.o.); Group 6 OUA + SNL (80mg/kg p.o.); Group 7 OUA + Li (60mg/kg, i.p), and Group 8 OUA + Li + SNL80. From the first to the 28th day, several behavioral parameters (Forced swim test, Open field test, Locomotor activity) were measured. The 28th day was marked by collecting biological samples (CSF and blood plasma) from Wistar adult rats. The animals were fully anesthetized with sodium pentobarbital (270 mg/ mL, i.p.), and then fresh brains were preserved in ice-cold PBS (0.1 M) of PBS for further biochemical evaluation. The biochemical estimation of SIRT-1 level determination in brain homogenate, blood plasma, and CSF was performed on the 29th and 30th days. Oxidative indicators (AChE, LDH, MDA, GSH, SOD, Nitrite) were also measured in brain homogenates. Similarly, apoptotic markers (Caspase-3, Bax, Bcl-2) and mitochondrial ETC-complexes enzymes (Complex-I, II, IV, V, and CoQ10) in the brain homogenate and blood plasma were also examined. Inflammatory markers (IL-1, TNF-) and neurotransmitters (Ach, Dopamine, 5-HT, Glutamate) were also measured in brain homogenate and blood plasma. The protocol for the experiment is summarized in (Fig. 1).

2.4 ICV-OUA induced experimental animal model of BD

The OUA-induced BD experimental model in rats was established using a well-known method (Valvassori et al., 2019). Three days of OUA-ICV injection (1mM/0.5µl) were given to the rats in the experiment. According to Valvassori et al., OUA generates neurological damage similar to that shown in an experimental animal model of BD. It is a valid model for examining pathophysiological alterations similar

The rats were habituated to the laboratory environment. After acclimatization, all animals in the experimental groups were anesthetized with ketamine (75 mg/kg, i.p.) before being placed in a stereotaxic frame (Sharma et al., 2021). After shaving the head and cutting a midline scalp incision, the skull was exposed. With the tooth bar set at 0 mm, each animal skin overlying the skull, as well as the coordinates for the striatum, must be precisely measured (AP-1.0mm; ML-2.5mm; DV-3.5mm) (Valvassori et al., 2019). Then, according to the protocol schedule, all animals in the experimental groups received OUA (1mM/0.5µl/5min/Unilateral/ICV injection) for three days (1st, 3rd, and 7th days). The infusion was administered manually, using a Hamilton syringe, through the burr holes drilled onto the skull surface. The injection rate in the experimental groups was0.5µl/5min, with the needle remaining in place for a further 1 minute before being progressively removed. The cannula is sealed with a detachable plastic ear pin. Before being sutured with an absorbable surgical suture connected to a sterile surgical needle, the hole was filled with dental cement.

Rats were housed individually in a polyacrylic cage that usually contained a warm cloth for post-operative care. Special attention was given to them until they regained spontaneous movement, which usually occurred 2–3 hours after anesthesia. The temperature in the room was kept at 25 ± 3°C. To avoid physical trauma after surgery, milk and glucose water were kept in the cages for 2–3 days. Gentamycin (35 mg/kg) was given intraperitoneally to rats for three days to prevent sepsis, and lignocaine gel was applied to the sutured area to relieve pain. Neosporin powder was dusted on them to prevent bacterial infection of the skin. Following surgery, the general health of the body and clinical symptoms such as dehydration were closely examined.

After seven days, rats continued to eat healthy food and drink plenty of water, and their spontaneous mobility returned, indicating that they had healed. The protocol drug SNL at 40 and 80mg and the standard drug Lithium alone and Lithium in combination with SNL80 mg/kg were administered chronically beginning on day 8th and continuing until day 28th. Behavioral parameters such as locomotor activity, open field test, and force swim test were carried out in accordance with the protocol schedule. After completing the protocol schedule, all animals were decapitated on days 29th and 30th, and their brains were removed to perform biochemical, inflammatory, and neurochemical assessments(Valvassor et al., 2021).

2.5 Parameters assessed

Measurement of body weight

According to the protocol schedule, body weight was measured on the 1st, 7th, 14th, 21st, and 28th days of the experiment (Valvassori et al., 2019.

Assessment of behavioral parameters

Open field test (OFT)

The animals exhibited manic-like behaviour after a single injection of OUA for three days (1st, 3rd, and 7th). The rat was placed in a cage on the first day and trained to explore an open field for 5 minutes. During the test, a camera monitored each rat's activities, including an increase in the number of crossings, rearings, and time spent in the centre. According to the protocol schedule, on days 1st, 7th, 14th, 21st, and 28th, an open field test was used to measure the number of crossings, rearings, and time spent in the centre in rats (Kumar et al., 2021).

Locomotor activity

Increased locomotor activity is a sign of manic-like behaviour (Nestler and Hyman 2010). The device uses photocells to detect motor activity. The animals were placed in the activity room for 3 minutes prior to the recording for habituation. On the 1st, 9th, 18th, and 27th days after ICV administrations, locomotion was assessed using an actophotometer (INCO (Instruments and Chemicals Private Limited), Haryana) for 5 minutes, and values were represented as counts per 5 minutes (Rahi et al., 2021).

Forced swimming test (FST)

A force swim test was used to evaluate the immobility time. Individual rats were placed in cylindrical tanks (height 50 cm; diameter 15 cm) with 30 cm of water at a temperature of 24 ± 1°C. A camera filmed the rat's movements for 5 minutes. During the training session, rats are exposed to the tank for 15 minutes on the first day and 5 minutes on the second day. The testing period for rats consists of a single 6-minute exposure, with the first 2 minutes serving as a habituation period. Each animal was tested for its depressive-like behaviour on days 1st, 9th, 18th, and 27th following ICV injection. The immobility time was recorded for 5 minutes during each session. When the rat stopped struggling and stayed motionless in an upright position in the water, only making slight movements to keep its head above the water, it was determined to be immobile (Rahi et al., 2021).

Neurochemical alterations evaluation

Collection and preparation of biological samples

On day 29th of the experiment, 2.5 ml of blood was collected from anesthetized rats through retro-bulbar puncture from the orbital venous plexus by inserting a capillary tube medially into the rat eye. Blood from the plexus was collected into a sterile Eppendorf tube via the capillary action through gentle rotation and retraction of the tube (Boynton et al., 2020). The blood samples were then centrifuged at 10,000×g for 15 minutes to separate the plasma, and the supernatant was carefully stored in a deep freeze (at -80o C) for further use.

Following blood collection, rats were deeply anesthetized with sodium pentobarbital (270mg/ml, i.p.) and subjected to caudal incision, translucent duramater was exposed, and a 30 gauge needle was gently placed at 30□ angle into the cisterna magna (Pegg et al., 2010). Approximately 100µL CSF was carefully ejected into a 0.5ml sterile Eppendorf tube using the suction pressure of a 1ml tuberculin syringe was frozen at 80□ C until analyzed ELISA (Rubio et al., 2011).

Immediately after CSF collection, rats were sacrificed by decapitation; whole brains were isolated from the skull with the utmost care, freshly weighed and washed with ice-cold, isotonic saline solution, and then homogenized with 0.1M (w/v) of chilled PBS (pH = 7.4). The rat brain homogenate was then centrifuged at 10,000×g for 15 minutes, the supernatant was separated, and the aliquots were preserved. The samples were deep-freezed at -80ºC to be used as and when required for various biochemical estimations.

Assessment of cellular and molecular markers

Measurement of SIRT-1 protein level

The level of SIRT-1 protein expression was measured using standard ELISA kits (E Lab Science, China). This test was carried out in the brain homogenate (Kumar et al., 2021), blood plasma (Wu et al., 2012), and CSF (David et al., 2015)according to the standard technique. The values are given in brain homogenate as nM/µg protein (Minj et al., 2021) and as ng/ml protein in blood plasma (Mariani et al., 2018) and CSF (Jamali-Raeufy et al., 2020).

Assessment of apoptotic markers

Measurement of caspase-3 level

Caspase-3 concentrations were determined using commercial ELISA kits (E Lab Science, China). ELISA kits were used to perform this test in brain homogenate (Rahi et al., 2021) and blood plasma (Guo et al., 2018).

Measurement of Bax and Bcl-2 levels

Commercial ELISA kits were used to determine the protein levels of Bax and Bcl-2 (E Lab Science, China). The level of Bax protein in brain homogenate (Tiwari et al., 2021) and blood plasma was measured (Wang et al., 2018). Using ELISA commercial kits, the quantities of anti-apoptotic proteins such as Bcl-2 were evaluated in brain homogenate (Sharma et al., 2019) and blood plasma (Wang et al., 2018). (Elabsciences, China).

Assessment of mitochondrial ETC-complexes enzyme levels

Preparation of Post mitochondrial supernatant (PMS) from rat whole-brain homogenate

The rat whole brain homogenate was centrifuged for 20 minutes at 5000 rpm at 4°C, and the resulting supernatant was used as rat brain PMS for further research. Differential centrifugation was used to prepare the crude mitochondrial fraction. By gently shaking at 4°C for 60 minutes, the pellet generated during the preparation of PMS was combined with 0.1M sodium phosphate buffer (pH 7.4) in a 1:10 proportion. The pellets were re-suspended in the same buffer containing extra sucrose at a concentration of 250 mmol/L after centrifugation at 16000 rpm at 0°C for 30 minutes. The centrifugation and

resuspension steps were done three times, and the crude mitochondrial fraction produced in the buffered sucrose solution was used for further investigation (Sharma et al., 2021; Rana et al., 2019).

Mitochondrial ETC complex-I enzyme activity (NADPH dehydrogenase)

To determine complex-I activity, the rate of NADH oxidation at 340 nm in an assay medium was measured spectrophotometrically at 37°C for 3 minutes. In the absence and presence of 2 µM rotenone, reactions were carried out, and the rotenone-sensitive activity was assigned to complex-I (Sharma et al., 2021; Mehan et al., 2020).

Mitochondrial ETC complex-II enzyme activity(Succinatedehydrogenase/SDH)

At 490nm(Shimadzu, UV-1700), the absorbance of a 0.3 mL sodium succinate solution in a 50µl gradient fraction of homogenate was measured. The molar extinction coefficient of the chromophore (1.36×104 M − 1 cm − 1) was used to determine the results, which were reported as INT decreased µmol/mg protein (Sharma et al., 2021; Kapoor et al., 2019).

Mitochondrial ETC complex-IV enzyme activity (cytochrome oxidase)

Reduced cytochrome-C (0.3 mM) was added to the assay mixture in a 75 mM phosphate buffer. The process was started by adding a solubilized mitochondrial sample, and the absorbance change was measured for 2 minutes at 550 nm (Sharma et al., 2021; Alam et al., 2019).

Mitochondrial ETC complex-V enzyme activity (ATP synthase)

To inactivate the ATPases, aliquots of homogenates were sonicated immediately in ice-cold perchloric acid (0.1N). Supernatants containing ATP were neutralized with 1N NaOH and kept at -80°C until analysis after centrifugation (14.000 g, 4°C, and 5 min). A reverse-phase HPLC was used to measure the amount of ATP in the supernatants (PerkinElmer). The reference solution of ATP was made according to the dissolving standard, and the detecting wavelength was 254 nm (Sharma et al., 2021; Dudi et al., 2018).

Assessment of neurotransmitters levels

Measurement of brain serotonin levels

The level of serotonin in brain homogenate was estimated using the method of Sharma et al. with minor modifications. HPLC with an electrochemical detector and a C18 reverse-phase column was used to determine it. Sodium citrate buffer (pH 4.5) – acetonitrile (87: 13, v/v) is used in the mobile phase. Ten mmol/L citric acid, 25 mmol/L NaH2 HPO4, 25 mmol/L EDTA, and two mmol/L 1- heptane sulfonic acid made up the sodium citrate buffer. The electrochemical parameters in the experiments were + 0.75 V, with sensitivity ranging from 5 to 50 nA. At a flow rate of 0.8 ml/min, the separation procedure was carried out. 20 µl of samples were manually injected. On the day of the experiment, brain samples were homogenized in 0.2 mol/L perchloric acid. The samples were then centrifuged for 5 minutes at 12,000

nm nylon filters before being injected into the HPLC sample

injector. With the help of the breeze program, data were collected and evaluated. Using a standard with a concentration of 10–100 mg/ml, serotonin concentrations were determined from the standard curve (Sharma et al., 2021).

Assessment of brain dopamine levels

Dopamine levels in striatal tissue samples were measured using Tiwari and colleague's technique. Dopamine activity in rat brain homogenate quantified as ng/mg protein (Tiwari et al., 2021).

Assessment of brain glutamate levels

According to the Alam et al., glutamate was measured in tissue samples after derivatization with o-phthalaldehyde/β-mercaptoethanol (OPA/β-ME) and quantitative analysis In rat brain homogenates, glutamate activity is reported as ng/mg protein (Alam et al., 2020).

Assessment of brain acetylcholine levels

A diagnostic kit is used to measure acetylcholine (Krishgen diagnostics, India). All reagents and rat brain homogenate were produced according to the kit's normal procedure. In the microtiter plate, the optical density of the reaction mixture was determined at 540 nm (Mehan et al., 2020).

Assessment of neuroinflammatory cytokines

Measurement of TNF- α and IL-1β levels

Using a rat immunoassay kit(KRISHGEN BioSystem, USA), the level of TNF-α was measured in rat brain homogenate (Mehan et al., 2018) and blood plasma. The activity of IL-1β was measured in rat brain homogenate and blood plasma as pg/mg protein (Tiwari et al., 2021).

Estimation of oxidative stress markers

Measurement of acetylcholinesterase (AChE) levels

The levels of acetylcholinesterase (AChE) were measured using spectrophotometry. The 0.05 ml supernatant, 3 ml 0.01M sodium phosphate buffer (pH 8), 0.10 ml acetylthiocholine iodide, and 0.10 ml DTNB were used in the test mixture (Ellman reagent). The absorbance change was spectrophotometrically recorded at 412nm right away.In the supernatant, the enzymatic activity is represented as μM/mg protein (Sharma et al., 2021).

Measurement of reduced glutathione levels

In the brain homogenate, the level of reduced glutathione was determined. 1 mL supernatant was precipitated with 1 mL 4% sulfosalicylic acid and cold digested for 1 hour at 4°C. The samples were centrifuged for 15 minutes at 1200 rpm. To 1 ml supernatant, 2.7 ml phosphate buffer (0.1M, pH 8) and TNB) were added. A spectrophotometer was used to measure

the yellow colour that emerged at 412nm right away. Glutathione content in the supernatant, given as µM/mg protein (Deshmukh et al., 2009).

Measurement of nitrite levels

A colorimetric assay utilizing Greiss reagent (0.1 % N-(1- naphthyl) ethylenediamine dihydrochloride, % sulfanilamide, and % phosphoric acid) determines the concentration of nitrite in the supernatant, which is indicative of the formation of nitric oxide (NO). Equal amounts of supernatant and Greiss reagent are mixed, the mixture is incubated at room temperature in the dark for 10 minutes, and the absorbance is measured spectrophotometrically at 540nm. A sodium nitrite standard curve is used to calculate nitrite concentration in the supernatant, which is given as µM/mg protein(Deshmukh et al., 2009).

Measurement of malondialdehyde (MDA) levels

The MDA end product of lipid peroxidation was determined quantitatively in brain homogenates. A spectrophotometer was used to measure the quantity of MDA after its reaction with thiobarbituric acid at 532nm. MDA concentration is expressed in nM/mg of protein (Mehan et al., 2011).

Measurement of superoxide dismutase (SOD) levels

SOD activity was evaluated by auto-oxidation of epinephrine at pH 10.4 using spectrophotometry. The brain homogenate supernatant (0.2 ml) was combined with 0.8 ml of 50 mM glycine buffer, pH 10.4, and the reaction was begun with 0.02 ml epinephrine. The absorbance was spectrophotometrically measured at 480nm after 5 minutes. The activity of SOD was measured in nM/mg of protein (Sharma et al., 2019).

Measurement of lactate dehydrogenase (LDH) assay

A diagnostic kit (Coral Diagnostics, India) was used to quantify the amount of LDH in the rat brain homogenate, and the amount of LDH was quantified as Units/L. (Khera et al., 2019).

Protein estimation

A Coral protein estimation kit (Biuret method) was used to determine the protein content.

Statistical analysis

The mean and standard error of the mean was used to express all of the findings (SEM). The data were analyzed using a two-way ANOVA followed by a Bonferroni post hoc test and a one-way ANOVA followed by a Tukey's multi comparison test. It was determined that $P < 0.001$ was statistically significant. The sample size was estimated after the data was confirmed to be normalized, and the normality distribution was checked using the Kolmogorov Smirnov test. GraphPad Prism version 5.03 for Windows was used to generate all statistical results (GraphPad Software, San Diego, CA, USA). The mean and standard error of the mean was used to express the statistical data (SEM).

3.0 Results

3.1 Neuroprotective potential of solanesol on weight variations in ouabain-induced bipolar disorder rats

Improvement in body weight after solanesol treatment

Bodyweight was measured once a week, on days 1st, 7th, 14th, 21st, and 28th of the procedure schedule. Figure 2 depicts the differences in body weight caused by the toxin OUA compared to the treatment drugs over the protocol schedule. Compared to the vehicle, sham, and SNL80 *perse* treated groups, the administration of OUA for 1st, 3rd, and 7th days resulted in a consistent decline in body weight. From day 8th to day 28th, rats receiving prolonged oral treatment with SNL and Lithium demonstrate a remarkable restoration in body weight due to improvements in psychiatric behaviors such as decreased locomotor activity, rearing, stress, and increased food intake.

Compared to SNL40 and SNL80 mg/kg treated rats, the Li60 mg/kg treated rats showed a more significant improvement in body weight. In addition, compared to other drug treatment groups such as SNL40 mg/kg, SNL80 mg/kg, and Li60 mg/kg; standard drug Li60 mg/kg in combination with SNL80 mg/kg showed significant weight restoration. SNL 80 mg/kg has been shown to be more effective than SNL40 mg/kg in recovering OUA-induced lower body weight, demonstrating that SNL has a dose-dependent impact on restoring body weight [Two-way ANOVA: $F(28, 160) = 903.4, p < 0.001$]. (Fig. 2)

3.2 Neuroprotective potential of solanesol in the prevention of neurobehavioral abnormalities in ouabain-induced bipolar disorder rats

Decrease manic-like behavior after solanesol treatment in the open field task

Three days (1st, 3rd, and 7th) following a single OUA injection, the animals developed manic-like behaviors, as seen by the increased number of crossings, rearings, and time spent in the centre. Open field parameters were conducted on days 1st, 7th, 14th, 21st, and 28th of the protocol period to determine the number of crossings, number of rearings, and time spent in the centre in rats.

1. *Decrease number of crossing after solanesol treatment*

The number of boxes crossed by rats in an open field is depicted in Fig. 3a. There was no significant difference between the groups on the 1st day. The OUA-treated rats crossed more boxes than the vehicle, sham, and SNL80-treated rats. On the 7th day, there was no significant difference between the OUA-treated group and the other treatment groups. After 20 days of oral administration of the neurotoxic OUA, the SNL treatment group had a progressive reduction in the number of boxes crossing compared to the normal control, vehicle control, and SNL80 *perse* groups on days 14th, 21st, and 28th. At the 21st and 28th days, the Li60 mg/kg alone and in combination with SNL80 mg/kg treated animals had

considerably reduced the number of boxes crossing than the SNL80 mg/kg and SNL40 mg/kg treated groups. Furthermore, when comparing SNL80 mg/kg treatment to SNL40 mg/kg treatment in BD rats, animals showed a lesser number of boxes crossed [Two-way ANOVA: $F(28,160) = 190.0$, $p < 0.001$]. (Fig. 3a)

1. *Decrease number of rearing after solanesol treatment*

In the open field, the number of rearing behaviors in BD rats is shown in Fig. 3b.On the 1st day, there was no significant difference between the groups. The OUA-treated rats showed more rearing moves than the vehicle control, sham control, and SNL80 treated rats. There was no significant difference between the OUA treated and other treatment groups on the 7th day. On days 14th, 21st, and 28th, after 20 days of oral administration of the OUA, the number of rearings in the SNL treated groups decreased over time compared to the normal control, vehicle control, and SNL80 *perse* groups. The Li60 mg/kg alone and Li60 mg/kg along with SNL80 mg/kg treated animals showed a significantly lesser number of rearing on 21st and 28th days than the SNL80 mg/kg and SNL40 mg/kg treated groups. Furthermore, when BD rats were given SNL80 mg/kg versus SNL40 mg/kg, the animals showed a lesser number of rearing movements. [Two-way ANOVA: $F(28,160) = 39.51$, $p < 0.001$]. (Fig. 3b)

1. *Decrease time spent in the centre after solanesol treatment*

Figure 3c indicates BD rats in the open field time spent in the centre. On the 1st day, there was no significant difference between the groups. The OUA-treated rats stayed longer than vehicle, sham, and SNL80-treated rats. There was no significant difference between the OUA-treated group and the other treatment groups on the seventh day. On days 14th, 21st, and 28th compared to the normal control, vehicle control, and SNL80 *perse* groups, time spent in the centre in the SNL treated groups reduced over time following 20days of oral administration of the OUA.The Li60 mg/kg alone and Li60 mg/kg combined with SNL80 mg/kg treated animals spent significantly less time in the centre on the 21st and 28th days than the SNL80 mg/kg and SNL40 mg/kg treated groups. Moreover, BD rats administered SNL80 mg/kg spent less time in the centre than rats given SNL40 mg/kg. [Two-wayANOVA: $F(28,160) = 27.00$, $p < 0.001$]. (Fig. 3c)

Decreased manic-like behavior after solanesol treatment

As illustrated in Fig. 4, the results suggest that OUA significantly affects locomotor activity in BD rats. On the 1st day, there was no significant difference between the groups. Rats were given OUA on days 1st, 3rd, and 7th, demonstrating considerably higher locomotor activity during the protocol schedule compared to the vehicle control, sham control, and SNL80 treated rats. Locomotor activity decreased from day 8th to day 28th after SNL treatment, as observed with the mood stabilizer Li60 mg/kg treated rats. Compared to the SNL80 mg/kg and SNL40 mg/kg treatment groups, Li60 mg/kg administration, both alone and in combination with SNL80 mg/kg, significantly reduced locomotor activity. In addition, SNL80 mg/kg significantly reduced locomotor activity in actophotometer rats when compared to SNL40 mg/kg treated [illegible]) = 244.1, $p < 0.001$]. These results indicate that Lithium and

SNL have an antimanic effect when given alone and a more significant enhancement in antimanic action when given together during OUA-induced BD rats on days 18th and 27th. (Fig. 4)

Decreased depression-like behavior after solanesol treatment

As shown in Fig. 5, the results reveal that OUA has a considerable influence on immobility time in BD rats. On the 1st day, there was no significant difference between the groups. Rats were given OUA on days 1st, 3rd, and 7th had significantly prolonged immobility time during the protocol schedule compared to the vehicle control, sham control, and SNL80 *perse* treated rats. From day 8th to day 28th, immobility time was significantly reduced with SNL treatment, as reported with the mood stabilizer Li60 mg/kg. Li60 mg/kg treatment, both alone and in combination with SNL80 mg/kg, significantly reduced immobility time compared to the SNL80 mg/kg and SNL40 mg/kg treatment groups. Furthermore, compared to SNL40 mg/kg treated rats on day 27th, SNL80 mg/kg significantly reduced immobility time in FST rats [Two-way ANOVA: $F(21,120) = 244.1$, $p < 0.001$]. Li60 mg/kg and SNL80 mg/kg showed an antidepressant effect when administered alone on day 27th in OUA-induced BD rats and a more significant effect when given in combination (Fig. 5)

3.3 Neuroprotective potential of solanesol on neurochemical alterations in ouabain-induced bipolar disorder rats

Increased SIRT-1 level after long-term administration of solanesol

At the end of the protocol schedule, SIRT-1 levels were measured in rat brain homogenate, blood plasma, and CSF samples. Compared to normal control, vehicle control, and SNL80 perse groups, the ICV injection of OUA resulted in a significant decline in SIRT-1 levels. The level of SIRT-1 in brain homogenate [One-way ANOVA: $F(7, 35) = 4.472$, $P < 0.001$], blood plasma [One-way ANOVA: $F(7, 35) = 5.938$, $P < 0.001$], and CSF [One-way ANOVA: $F(7, 35) = 1.243$, $P < 0.001$] samples were elevated after continuous oral administration of SNL at doses of 40 mg/kg and 80 mg/kg. In rat brain homogenate, blood plasma, and CSF samples, SNL80 mg/kg was more effective than SNL40 mg/kg in restoring SIRT-1 protein expression. Furthermore, the Li60 mg/kg alone and Li60 mg/kg in combination with SNL80 mg/kg treated groups were more effective in restoring SIRT-1 protein expression in rat brain homogenate, blood plasma, and CSF samples than the SNL80 mg/kg and SNL40 mg/kg treated groups. (Table 1)

Table 1
Neuroprotective potential of solanesol on SIRT-1 level in ouabain-induced bipolar disorder in rats

S.no.	Groups	SIRT-1		
		Brain homogenate (nM/µg protein)	Blood plasma (ng/ml)	CSF (ng/ml)
1.	Vehicle control	311.20 ± 5.164	6.07 ± 0.074	3.29 ± 0.073
2.	Sham control	312.30 ± 5.102	6.07 ± 0.105	3.33 ± 0.047
3.	SNL80 *perse*	311.90 ± 4.278	6.00 ± 0.081	3.26 ± 0.052
4.	OUA	153.20 ± 9.224*	2.43 ± 0.100*	0.80 ± 0.065*
5.	OUA + SNL40	180.50 ± 2.832#	3.28 ± 0.071#	1.35 ± 0.048#
6.	OUA + SNL80	210.50 ± 3.103#$	3.79 ± 0.074#$	1.64 ± 0.045#$
7.	OUA + Li60	237.60 ± 3.616#β	4.29 ± 0.066#β	1.92 ± 0.041#β
8.	OUA + SNL80 + Li60	267.40 ± 2.215#@	4.77 ± 0.077#@	2.25 ± 0.036#@

Statistical analysis followed by one-way ANOVA (post-hoc Tukey's test). Values expressed as mean ± SEM (n = 6 rats per group). * $p < 0.001$ v/s vehicle control, sham control and SNL80 perse; # $p < 0.001$ v/s OUA; #$ $p < 0.001$ v/s OUA + SNL40; #β $p < 0.001$ v/s OUA + SNL40 and OUA + SNL80; #@ OUA + Li60

Decreased level of caspase-3, Bax, and increased Bcl-2 levels after long-term administration of solanesol

The levels of cell death indicators such as Caspase-3, Bax, and Bcl-2 were measured in rat brain homogenate and blood plasma samples after the protocol schedule. In rat brain homogenate and blood plasma samples, ICV injection of OUA treatment resulted in a significant increase in pro-apoptotic markers such as caspase-3 and Bax. In contrast, the ICV injection of OUA for three days (1st, 3rd, and 7th) resulted in a significant decrease in anti-apoptotic Bcl-2 protein levels in rat brain homogenate and blood plasma samples compared to the normal control, vehicle control, and SNL80 perse treated groups. Chronic oral treatment of SNL40 mg/kg and SNL80 mg/kg significantly lowered caspase-3 levels in brain homogenate [One-way ANOVA: $F(7, 35) = 0.522$, $P < 0.001$] and blood plasma samples [One-way ANOVA: $F(7, 35) = 1.739$, $P < 0.001$] respectively.

Similarly, continuous oral administration of SNL40 mg/kg and 80 mg/kg significantly reduced the amount of pro-apoptotic Bax in rat brain homogenate [One-way ANOVA: $F(7, 35) = 1.092$, $P < 0.001$] and blood plasma samples [One-way ANOVA: $F(7, 35) = 1.628$, $P < 0.001$].

Furthermore, regular oral administration of SNL at doses of 40 mg/kg and 80 mg/kg for 20 days (day 8th to 28th) resulted in a significant rise in Bcl-2 protein levels in brain homogenate [One-way ANOVA: $F(7, 35) = 1.225$, $P < 0.001$] and blood plasma [One-way ANOVA: $F(7, 35) = 1.968$, $P < 0.001$] samples with

respect to the OUA-treated BD rats. Also, SNL80 mg/kg treatment was more effective than SNL40 mg/kg treatment in restoring abnormal levels of apoptotic markers in BD rats. Furthermore, in rat brain homogenate and blood plasma, the Li60 mg/kg alone and Li60 mg/kg combined with SNL80 mg/kg treated groups showed more significance in restoring the altered levels of apoptotic markers than the SNL80 mg/kg and SNL40 mg/kg treated groups. (Table 2)

Table 2
Neuroprotective potential of solanesol on Caspase-3, Bax, and Bcl-2 level in ouabain-induced bipolar disorder in rats

S. no.	Groups	Apoptotic markers					
		Caspase-3		Bax		Bcl-2	
		Brain homogenate (nM/mg protein)	Blood plasma (ng/ml)	Brain homogenate (ng/mg protein)	Blood plasma (ng/ml)	Brain homogenate (ng/mg protein)	Blood plasma (ng/ml)
1.	Vehicle control	89.96 ± 0.861	1.71 ± 0.028	6.60 ± 0.190	0.90 ± 0.061	26.77 ± 0.133	6.44 ± 0.049
2.	Sham control	90.07 ± 0.819	1.68 ± 0.020	6.73 ± 0.126	0.90 ± 0.058	26.65 ± 0.144	6.51 ± 0.070
3.	SNL80 *perse*	90.18 ± 0.947	1.69 ± 0.029	6.62 ± 0.125	0.86 ± 0.061	26.57 ± 0.177	6.49 ± 0.044
4.	OUA	132.1 0 ± 0.717*	4.79 ± 0.073*	11.76 ± 0.089*	4.58 ± 0.062*	18.80 ± 0.117*	1.70 ± 0.072*
5.	OUA + SNL40	117.90 ± 0.677$^{\#}$	3.71 ± 0.075$^{\#}$	10.67 ± 0.074$^{\#}$	4.07 ± 0.061$^{\#}$	21.54 ± 0.147$^{\#}$	2.79 ± 0.063$^{\#}$
6.	OUA + SNL80	112.80 ± 0.779$^{\#\$}$	3.29 ± 0.067$^{\#\$}$	9.79 ± 0.074$^{\#\$}$	3.52 ± 0.061$^{\#\$}$	22.81 ± 0.106$^{\#\$}$	3.62 ± 0.077$^{\#\$}$
7.	OUA + Li60	108.10 ± 0.812$^{\#\beta}$	2.78 ± 0.069$^{\#\beta}$	8.70 ± 0.068$^{\#\beta}$	2.38 ± 0.061$^{\#\beta}$	23.79 ± 0.118$^{\#\beta}$	4.57 ± 0.077$^{\#\beta}$
8.	OUA + SNL80 + Li60	102.40 ± 0.793$^{\#@}$	2.29 ± 0.064$^{\#@}$	7.78 ± 0.074$^{\#@}$	1.61 ± 0.040$^{\#@}$	24.83 ± 0.106$^{\#@}$	5.32 ± 0.045$^{\#@}$

Statistical analysis followed by one-way ANOVA (post-hoc Tukey's test). Values expressed as mean ± SEM (n = 6 rats per group). * $p < 0.001$ v/s vehicle control, sham control and SNL80 perse; # $p < 0.001$ v/s OUA; #$ $p < 0.001$ v/s OUA + SNL40; #β $p < 0.001$ v/s OUA + SNL40 and OUA + SNL80; #@ OUA + Li60

Restoration of mitochondrial ETC-complexes enzyme level after long-term administration of solanesol

After the experiment protocol schedule, the enzyme activity of mitochondrial ETC-complexes was

ys intoxications of OUA in rats through ICV injection resulted

in a significant decrease in mitochondrial ETC complexes-I [One-way ANOVA: $F(7, 35) = 1.796$, $P < 0.001$], complexes-II [One-way ANOVA: $F(7, 35) = 2.936$, $P < 0.001$], complexes-IV [One-way ANOVA: $F(7, 35) = 6.744$, $P < 0.001$], and complexes-V [One-way ANOVA: $F(7, 35) = 0.979$, $P < 0.001$] and CoQ10 level [One-way ANOVA: $F(7, 35) = 4.381$, $P < 0.001$], when compared to the vehicle, sham control, and SNL80 perse groups.

In OUA-treated rats, twenty days of chronic administration with SNL40mg/kg and SNL80 mg/kg substantially and dose-dependently recovers and increases mitochondrial ETC complex enzymatic activity. The significant restoration was observed with a high dose of SNL80 mg/kg group in mitochondrial ETC complexes-I, II, IV, V, and CoQ10 compared to a low dose of SNL40 mg/kg. The most significant improvements in mitochondrial ETC complexes-I, II, IV, V, and CoQ10 in rat brain homogenate were seen in the Li60 mg/kg alone and Li60 mg/kg in combination with SNL80 mg/kg treated groups, which were more effective than the SNL80 mg/kg and SNL40 mg/kg treated groups. (Table 3)

Table 3
Neuroprotective potential of solanesol on TNF-α and IL-1β level in ouabain-induced bipolar disorder in rats

S. no.	Groups	Neuroinflammatory markers			
		TNF-α (pg/mg protein)		IL-1β (pg/mg protein)	
		Brain homogenate (nM/mg protein)	Blood plasma (ng/ml)	Brain homogenate (ng/mg protein)	Blood plasma (ng/ml)
1.	Vehicle control	28.16 ± 0.594	20.67 ± 0.330	14.52 ± 0.143	14.39 ± 0.248
2.	Sham control	28.18 ± 0.535	20.94 ± 0.314	14.47 ± 0.126	14.15 ± 0.219
3.	SNL80 *perse*	28.53 ± 0.542	20.97 ± 0.324	14.49 ± 0.113	14.50 ± 0.240
4.	OUA	61.02 ± 0.827*	96.21 ± 1.371*	26.15 ± 0.151*	77.49 ± 0.560*
5.	OUA + SNL40	53.15 ± 0.778$^{\#}$	72.76 ± 1.096$^{\#}$	22.61 ± 0.055$^{\#}$	57.12 ± 0.608$^{\#}$
6.	OUA + SNL80	46.80 ± 0.723$^{\#\$}$	57.51 ± 0.648$^{\#\$}$	21.79 ± 0.067$^{\#\$}$	42.34 ± 0.609$^{\#\$}$
7.	OUA + Li60	40.86 ± 0.745$^{\#\beta}$	43.84 ± 0.502$^{\#\beta}$	20.70 ± 0.068$^{\#\beta}$	26.74 ± 0.454$^{\#\beta}$
8.	OUA + SNL80 + Li60	35.77 ± 0.745$^{\#@}$	27.70 ± 0.502$^{\#@}$	19.67 ± 0.051$^{\#@}$	19.30 ± 0.313$^{\#@}$

Statistical analysis followed by one-way ANOVA (post-hoc Tukey's test). Values expressed as mean ± SEM (n = 6 rats per group). * $p < 0.001$ v/s vehicle control, sham control and SNL80 perse; # $p < 0.001$ v/s OUA; #$ $p < 0.001$ v/s OUA + SNL40; #β $p < 0.001$ v/s OUA + SNL40 and OUA + SNL80; #@ OUA + Li60

Restoration of neurotransmitter level after long-term administration of solanesol

Neurochemicals such as serotonin, dopamine, glutamate, and acetylcholine were analysed in rat brain homogenate samples at the end of the experimental protocol schedule. The injection of OUA through the ICV route considerably reduced serotonin and acetylcholine levels.ICV injection of OUA intoxication resulted in a significant increase in dopamine and glutamate concentrations in brain homogenate compared to normal control, vehicle control, and SNL80 perse treated rats. Treatment with SNL40 mg/kg and 80 mg/kg significantly and dose-dependently increased serotonin [One-way ANOVA: $F(7, 35) = 4.031$, $P < 0.001$] as well as acetylcholine level [One-way ANOVA: $F(7, 35) = 3.607$, $P < 0.001$]. In contrast to the OUA-treated BD rats, prolonged oral administration of SNL40 mg/kg and SNL80 mg/kg decreased the

concentrations of dopamine [One-way ANOVA: F(7, 35) = 1.000, P < 0.001] and glutamate [One-way ANOVA: F(7, 35) = 1.963, P < 0.001] in rat brain homogenate. Moreover, SNL80 mg/kg versus SNL40 mg/kg treated rats re-establish lower neurotransmitter levels. The Li60 mg/kg alone and Li60 mg/kg combined with SNL80 mg/kg treated groups were more effective than the SNL80 mg/kg and SNL40 mg/kg treated groups in restoring the altered levels of neurotransmitters in rat brain homogenate. (Table 4)

Table 4
Neuroprotective potential of solanesol on neurotransmitters level in ouabain-induced bipolar disorder in rats

S. no.	Groups	Neurotransmitters			
		Serotonin (ng/mg protein)	Acetylcholine (ng/mg protein)	Glutamate (ng/mg protein)	Dopamine (ng/mg protein)
1.	Vehicle control	35.69 ± 0.413	6.63 ± 0.121	92.13 ± 1.413	77.13 ± 1.332
2.	Sham control	35.64 ± 0.516	6.54 ± 0.120	92.15 ± 1.305	77.21 ± 1.215
3.	SNL80 *perse*	35.59 ± 0.444	6.62 ± 0.147	92.05 ± 1.492	78.10 ± 1.228
4.	OUA	13.46 ± 0.527*	0.52 ± 0.114*	240.60 ± 1.808*	63.49 ± 0.967*
5.	OUA + SNL40	17.69 ± 0.430$^{\#}$	1.78 ± 0.079$^{\#}$	195.30 ± 1.502$^{\#}$	55.16 ± 0.640#
6.	OUA + SNL80	21.92 ± 0.446$^{\#\$}$	2.83 ± 0.084$^{\#\$}$	165.30 ± 1.412$^{\#\$}$	45.61 ± 0.566$^{\#\$}$
7.	OUA + Li60	25.66 ± 0.452$^{\#\beta}$	3.78 ± 0.077$^{\#\beta}$	136.40 ± 1.473$^{\#\beta}$	37.83 ± 0.765$^{\#\beta}$
8.	OUA + SNL80 + Li60	30.01 ± 0.446$^{\#@}$	4.78 ± 0.077$^{\#@}$	116.30 ± 1.487$^{\#@}$	27.22 ± 1.897$^{\#@}$

Statistical analysis followed by one-way ANOVA (post-hoc Tukey's test). Values expressed as mean ± SEM (n = 6 rats per group). * p < 0.001 v/s vehicle control, sham control and SNL80 perse; # p < 0.001 v/s OUA; #$ p < 0.001 v/s OUA + SNL40; #β p < 0.001 v/s OUA + SNL40 and OUA + SNL80; #@ OUA + Li60

Reduction in neuroinflammatory cytokines after long-term administration of solanesol

We measured the levels of pro-inflammatory cytokines like TNF-α and IL-1β in the whole brain homogenate and blood plasma samples of rats to see whether SNL had a therapeutic effect in OUA-induced BD rats. SNL therapy at doses of 40 mg/kg and 80 mg/kg reduced TNF-αexpression in rat brain homogenate [One-way ANOVA: F(7, 35) = 1.065, P < 0.001] and blood plasma samples [One-way ANOVA: F(7, 35) = 0.589, P < 0.001]. Similarly, chronic oral treatment with SNL40 mg/kg and SNL80 mg/kg remarkably decreased the level of IL-1β in brain homogenate [One-way ANOVA: F(7, 35) = 0.348, P < 0.001]

and blood plasma samples [One-way ANOVA: F(7, 35) = 0.691, P < 0.001], as opposed to the OUA toxin administered BD rats. Meanwhile, compared to the SNL40 mg/kg dose, SNL80 mg/kg demonstrated a significant improvement in lowering the expression of these inflammatory mediators. In rat brain homogenate and blood plasma samples, the Li60 mg/kg alone and Li60 mg/kg in conjunction with SNL80 mg/kg treated groups exhibited a substantial improvement in lowering the level of these inflammatory mediators compared to the SNL80 mg/kg and SNL40 mg/kg treated groups at the end of protocol schedule. (Table 5)

Table 5
Neuroprotective potential of solanesol in restoration of mitochondrial ETC complex enzymes in ouabain-induced bipolar disorder in rats

S. no.	Groups	Mitochondrial complexes estimation				
		Complex-I (nM/mg protein)	Complex-II (nM/mg protein)	Complex-IV (nM/mg protein)	Complex-V (nM/mg protein)	CoQ10 (nM/mg protein)
1.	Vehicle control	9.71 ± 0.077	11.77 ± 0.088	211.10 ± 1.505	450.40 ± 3.675	9.28 ± 0.240
2.	Sham control	9.73 ± 0.063	11.83 ± 0.089	210.70 ± 1.173	451.20 ± 2.648	9.08 ± 0.263
3.	SNL80 *perse*	9.77 ± 0.082	11.85 ± 0.083	209.80 ± 1.573	449.20 ± 3.251	9.00 ± 0.305
4.	OUA	4.33 ± 0.053*	3.52 ± 0.141*	118.00 ± 0.740*	160.60 ± 3.673*	2.03 ± 0.051*
5.	OUA + SNL40	5.74 ± 0.078$^{\#}$	5.28 ± 0.071$^{\#}$	130.30 ± 1.366$^{\#}$	210.20 ± 2.504$^{\#}$	3.28 ± 0.084$^{\#}$
6.	OUA + SNL80	6.77 ± 0.070$^{\#\$}$	6.29 ± 0.058$^{\#\$}$	149.70 ± 1.558$^{\#\$}$	269.00 ± 3.111$^{\#\$}$	4.29 ± 0.070$^{\#\$}$
7.	OUA + Li60	7.72 ± 0.080$^{\#\beta}$	7.33 ± 0.052$^{\#\beta}$	170.40 ± 1.527$^{\#\beta}$	342.20 ± 3.014$^{\#\beta}$	5.29 ± 0.078$^{\#\beta}$
8.	OUA + SNL80 + Li60	8.75 ± 0.079$^{\#@}$	8.20 ± 0.074$^{\#@}$	190.00 ± 1.449$^{\#@}$	391.00 ± 3.117$^{\#@}$	6.28 ± 0.057$^{\#@}$

Statistical analysis followed by one-way ANOVA (post-hoc Tukey's test). Values expressed as mean ± SEM (n = 6 rats per group). * p < 0.001 v/s vehicle control, sham control and SNL80 perse; # p < 0.001 v/s OUA; #$ p < 0.001 v/s OUA + SNL40; #β p < 0.001 v/s OUA + SNL40 and OUA + SNL80; #@ OUA + Li60

Decreased oxidative stress markers and increased antioxidant levels after long-term administration of solanesol

The levels of oxidative stress indicators such as AchE, LDH, MDA, nitrite, SOD, and GSH were measured in rat brain homogenate samples at the end of the experimental protocol schedule. The levels of AchE, LDH, MDA, and nitrite increased significantly after ICV injection of OUA. In contrast, antioxidant levels such as SOD and GSH decreased compared to the normal control, vehicle control, and SNL80 perse treated groups. Continuous oral treatment of SNL at doses of 40 mg/kg and 80 mg/kg for twenty days significantly lowered the levels of AchE [One-way ANOVA: $F(7, 35) = 2.867$, $P < 0.001$], LDH [One-way ANOVA: $F(7, 35) = 2.829$, $P < 0.001$], MDA [One-way ANOVA: $F(7, 35) = 3.681$, $P < 0.001$] and nitrite [One-way ANOVA: $F(7, 35) = 1.736$, $P < 0.001$].

However, SNL40 mg/kg and SNL80 mg/kg remarkably restored the anti-oxidant defense system by increasing the levels of GSH [One-way ANOVA: $F(7, 35) = 4.281$, $P < 0.001$], and SOD [One-way ANOVA: $F(7, 35) = 6.111$, $P < 0.001$] when compared with OUA-treated BD rats. Furthermore, in comparison to SNL40 mg/kg, SNL80 mg/kg significantly reduced oxidative stress markers and restored antioxidant expression in a dose-dependent manner. Among these, the most significant improvements were observed in the Li60 mg/kg alone and Li60 mg/kg in combination with SNL80 mg/kg treated groups, which were more effective than the SNL80 mg/kg and SNL40 mg/kg treated groups in significantly reducing oxidative stress markers and restoring antioxidant expression. (Table 6)

Table 6
Neuroprotective potential of solanesol on oxidative stress markers level in ouabain-induced bipolar disorder in rats

S.no.	Groups	Oxidative stress markers					
		AchE (\varvec$\mu$M/mg protein)	LDH (µM/mg protein)	SOD (µM/mg protein)	GSH (µM/mg protein)	Nitrite (µM/mg protein)	MDA (nM/mg protein)
1.	Vehicle control	18.61 ± 0.618	100.40 ± 1.523	390.30 ± 1.431	29.97 ± 0.781	5.28 ± 0.075	27.87 ± 0.665
2.	Sham control	17.82 ± 0.523	101.00 ± 1.560	389.70 ± 1.452	29.99 ± 0.785	5.32 ± 0.050	27.89 ± 0.519
3.	SNL80 *perse*	18.38 ± 0.545	100.10 ± 1.155	390.30 ± 1.621	29.98 ± 0.721	5.21 ± 0.065	27.90 ± 0.818
4.	OUA	45.11 ± 0.639*	326.60 ± 1.423*	268.60 ± 1.532*	8.21 ± 0.594*	10.29 ± 0.069*	61.37 ± 0.577*
5.	OUA + SNL40	39.83 ± 0.404$^{\#}$	296.10 ± 1.538$^{\#}$	285.50 ± 1.404$^{\#}$	14.08 ± 0.346$^{\#}$	9.22 ± 0.071$^{\#}$	52.76 ± 0.796$^{\#}$
6.	OUA + SNL80	34.52 ± 0.480$^{\#\$}$	246.00 ± 1.511$^{\#\$}$	315.40 ± 1.630$^{\#\$}$	17.27 ± 0.349$^{\#\$}$	8.24 ± 0.056$^{\#\$}$	45.15 ± 0.618$^{\#\$}$
7.	OUA + Li60	29.66 ± 0.442$^{\#\beta}$	195.40 ± 1.519$^{\#\beta}$	345.20 ± 1.262$^{\#\beta}$	20.24 ± 0.275$^{\beta}$	7.21 ± 0.586$^{\#\beta}$	37.95 ± 0.721$^{\#\beta}$
8.	OUA + SNL80 + Li60	24.73 ± 0.457$^{\#@}$	144.80 ± 1.337$^{\#@}$	375.80 ± 1.423$^{\#@}$	23.31 ± 0.297$^{\#@}$	6.21 ± 0.071$^{\#@}$	31.14 ± 0.612$^{\#@}$

Statistical analysis followed by one-way ANOVA (post-hoc Tukey's test). Values expressed as mean ± SEM (n = 6 rats per group). * $p < 0.001$ v/s vehicle control, sham control and SNL80 perse; # $p < 0.001$ v/s OUA; #$ $p < 0.001$ v/s OUA + SNL40; #β $p < 0.001$ v/s OUA + SNL40 and OUA + SNL80; #@ OUA + Li60

4.0 Discussion

During the past decade, there has been significant progress in understanding the role of sirtuins in brain aging, neurodegenerative disorders such as AD, PD, MS, ALS, and neuropsychiatric disorders such as BD (Jęśko and Strosznajder, 2016; Yuan et al., 2016). Till now, relatively little is known about the role of SIRTs in PD (Tang, 2016), HD (Duan, 2013), and MS (Sharma et al., 2021). SIRT-1 expression and activity may significantly affect the course of AD pathology and may be a promising therapeutic target (Kupis et al., 2016). SIRT-1 inhibition impairs behavioral impairment, neurochemical changes, and brain cell damage (Shah et al., 2017; Lima et al., 2017). SIRT-1 downregulation involves various events related to higher brain dysfunction, including synaptic dysfunction, altered neurotransmitter secretion, and genetic variants

(Fujita Yamashita, 2018). A previous study reported that no pharmacological animal model mimics mania and depression in the same animals (Logan et al., 2016). But Valvassori et al. established a model of single ICV injection of OUA elicited manic and depressive-like behaviour. The animals were treated to forced swimming in order to assess depressive-like behaviour (Valvassori et al., 2019).

Manic behaviours in rats, such as increased locomotor activity, rearing, and crossing, have been observed in several studies following ICV injection of OUA (Lopes-Borges et al., 2015; Valvassori et al., 2017). We discovered the same thing in our present study. The locomotor activity, number of boxes crossed, number of rearing movements, and time spent at the centre increased significantly after three days (1st, 3rd, and 7th) of protocol treatment in ICV-OUA induced BD rats. Also, the current study demonstrated manic and depressive-like behaviours in the same animal following a single OUA administration. As a result, the purpose of this study is to demonstrate OUA-induced contradictory behaviours in the same animal, and the SNL can prevent the BD-like behavioral, neurochemical and morphological alterations in OUA-induced BD in rats via upregulation of SIRT-1 signaling.

Compared to the normal control, vehicle control, and SNL80 perse groups, the animals did not show any behavioral changes in the open field test, forced swimming, and locomotor activity after OUA administration. As a result, it's possible that nine days following OUA injection, rats experience a calm episode (Valvassori et al., 2019; Wang et al., 2018; Kirshenbaum et al., 2011). The variance between experiments could be explained by differences in rat strains and experimental conditions. In the current study, the protocol was repeated for biochemical analysis, and we obtained identical results in the open-field test, with no behavioral changes. The concept of euthymia includes a stable period with no mood changes and an intra-state interval during which the patient does not exhibit enough mood symptoms to be categorized in a specific mood episode (Fava & Bech, 2016).

Seven days of lithium pretreatment reduced OUA-induced manic-like behaviour. Several investigations from our group and others show that lithium therapy can correct manic-like behaviour in rats subjected to OUA-ICV injection (El-Mallakh et al., 2003, Jornada et al., 2011). Lithium administration, on the other hand, partially reversed the immobility time. Although earlier preclinical studies have revealed lithium antidepressant effects (Silva et al., 2008; Mohsen et al., 2017), and current investigation mimicked the maintenance treatment of depression and manic-like behaviours in a prospective BD animal model.

The construction of an animal model of BD produced by OUA is based on the hypothesis that a decrease in Na+/K+-ATPase activity is critical in the onset of manic and depressed mood episodes (Valvassori et al., 2019; Riegel et al., 2009). To validate that the dose of OUA used here reduces Na+/K+-ATPase activity, this enzyme activity was measured in the brains of rats. OUA lowered Na+/K+-ATPase activity in the whole brain of the mice seven and nine days after ICV injection. More than 50 years ago, the idea of Na+/K+-ATPase in BD physiopathology was proposed (Vitezić et al., 2008). A meta-analysis study found that Na+/K+-ATPase activity is reduced in BD patients' erythrocytes (Omar et al., 2017). Even a minor decrease in this enzyme activity may bring the resting membrane potential close to the threshold, increasing neuronal excitability and slowing the rate of Ca2 + depuration (Lu et al., 2010). Hyperactivity,

which defines manic episodes in BD, could be triggered by increased neural excitability. Long-term Na+/K+-ATPase inhibition, which increases neuronal excitability, may decrease resting potential regulation, making subsequent neuronal depolarization more difficult. These events may diminish neuronal transmission velocity and, as a result, synaptic effectiveness of neurons, resulting in BD depressive episodes (Herman et al., 2007). Enhancement of Na+/K+ -ATPase activity could be one of lithium's therapeutic methods against oxidative damage. Oxidative damage was reported in rats given OUA, resembling pathophysiological features in BD patients. Indeed, decreased anti-oxidant glutathione enzymes in the brain have been found in animal models of mania and depression (Budni et al., 2013). Modulation of these anti-oxidant enzymes, which contributes to preserving redox equilibrium in the brain, is one of the lithium's probable therapeutic activities (Muneer et al., 2016). According to one study, decreased activity of Na+/K+-ATPase found in BD patients could be linked to increased secretion of dopamine and glutamate hormones as well as oxidative damage, resulting in mood swings (Sigitova et al., 2017).

As a mood stabilizer, lithium works to reverse these pathological alterations, which helps to alleviate BD symptoms. The proposed OUA model could be employed to research the disorder's pathogenesis and the screening of promising mood stabilizer medication candidates. Chronic treatment of OUA to the brain resulted in decreased ATP cell generation, increased oxidative stress-mediated by ROS and RNS, glial cell overactivation, and lower regulation of SIRT-1 protein, in addition to reduced ATP cell production (Valvassori et al., 2019). SIRT-1 deacetylation depends on NAD + and ATP synthesis in cells and regulates its amount in mitochondria and other parts of the brain. Memory impairment is also caused by SIRT-1 dysregulation, and oxidative markers have been utilized to identify the excessive production of ROS and RNS in the brain (Shin et al., 2015). In bipolar patients, an increase in oxidative stress has been linked to a decrease in the activity of the Na+/K+-ATPase (Valvassori et al., 2015).

According to current research, OUA-treated rats saw decreased independent body weight on days 14th, 21st, and 28th. Furthermore, there was an increase in locomotor activity in the actophotometer on days 9th, 18th, and 27th, responsible for manic-like behaviour. OFT observed this manic-like activity on days 7th, 14th, 21st, and 28th, indicating a progressive increase in the number of rearing, number of boxes crossing, and time spent in the center. Further FST on the 9th, 18th, and 27th days indicated an improvement in immobility time.

The effect of OUA on the protein level of SIRT-1 in the brain is being explored in this study. This suggests a decrease in SIRT-1 protein levels. Furthermore, the levels of apoptotic markers Caspase-3, Bax, and Bcl-2 were evaluated, and OUA-treated rats had higher levels of caspase-3, Bax, and lower levels of Bcl-2. In contrast, the downregulation of mitochondrial ETC complex enzymes has been linked to a significant increase in inflammatory cytokines TNF-α and IL-1β. The study shows that when rats are persistently treated with OUA, the amount of neurotransmitters changes. Neurotoxic effects in rats are indicated by decreased serotonin and acetylcholine levels and increased dopamine and glutamate levels. Oxidative damage is a major factor in neurodegenerative disorders. OUA treatment causes a significant increase in [redacted]reasing SOD and GSH.

Our research found that twenty days of chronic treatment of SNL40, 80 mg/kg in ICV injection to OUA-treated rats, resulted in a considerable improvement in body weight by the 21st day of the protocol. Furthermore, there was a 5-minute decrease in locomotor activity in the actophotometer. The high dose-response of SNL indicates a considerable reduction in behavioural activities, and the standard drug lithium alone and in combination with SNL high dose also exhibited a marked decline in behavioural parameters activity than SNL treated rats.

SIRT-1 levels rise after chronic treatment of SNL40 and SNL80 mg/kg, and SIRT-1 is activated in CSF, brain homogenate, and blood plasma samples, according to this study. In addition, Li-treated groups were more efficient than SNL-treated groups in recovering SIRT-1 protein expression in rat brain homogenate, blood plasma, and CSF samples. However, in blood plasma and brain homogenate, the apoptotic marker level reveals a drop in caspase-3, Bax, and a rise in Bcl-2. Furthermore, results reveal that continuous SNL treatment restores mitochondrial complexes such as Complex I, II, IV, and V and CoQ10 in brain homogenate. TNF-α and IL-1β levels in blood plasma and rat brain homogenate samples suggest that SNL treatment reduces neuronal inflammation. In addition, at all doses, SNL boosted serotonin and acetylcholine levels while decreasing dopamine and glutamate levels in rat brain homogenate.

Oxidative damage in OUA-treated rats treated with SNL40 and 80 mg/kg, on the other hand, reflects a reduction in oxidative stress through a significant drop in the levels of AchE, MDA, LDH, and nitrite levels. As well as a considerable increase in the quantity of anti-oxidant markers SOD and GSH in brain homogenate. In blood plasma and bairn homogenate samples, the Li60 mg/kg alone and Li60 mg/kg in conjunction with SNL80 mg/kg treated groups were more efficient than the SNL80 mg/kg SNL40 mg/kg treated groups in restoring the altered levels of biochemical parameters.

As a result, the current work finds that following ICV-OUA insertion in rats, SIRT-1 signaling and neuronal death are reduced. In addition, there was a depletion of mitochondrial ETC complexes in the case of the disease and an increase in inflammation and oxidative stress. Chronic treatment with SNL and Li causes changes and results in significant dose-dependent restorations. Consequently, these SIRT-1 presences and their SNL activators had a neuroprotective impact on OUA-mediated BD rat model ICV injections.

Although the current data are only associations, they show that SNL reduced the downregulation of SIRT-1 signaling in rats with BD-like behavioral and neurochemical symptoms in OUA-induced BD. Our results indicate that SIRT-1 levels can be employed for predicting a major brain degenerative component in brain tissue, blood plasma, and CSF as an effective and reliable early diagnostic biomarker. Lithium works as a mood stabilizer medicine to counteract these pathological alterations that help mitigate symptoms of BD. The proposed OUA model could be applied to investigate disease pathophysiology and the screening of prospective medication candidates for mood stabilizers.

Overall, a mechanistic approach must be verified using knock-in or knock-out examinations of the sirtuin genes. Additionally, correlative analysis, such as Western Blot for cellular markers, is required to provide molecular support for this idea. Even following these disadvantages, the potential in brain

downregulatory SIRT-1 signaling cascades of the neuroprotective component of SNL has been identified to develop a new disease-modifying medication for this neurodegenerative disease.

5.0 Conclusion

In conclusion, the study shows that SNL protects rats from developing BD caused by OUA. This is the first study to link the antioxidants, anti-inflammatory, and anti-apoptotic capabilities of SNL to its potential neuroprotective effect as a drug for the treatment and management of BD. The amount of different neurochemicals in brain homogenate, blood plasma, and CSF was measured, demonstrating that SNL had a protective effect both centrally and peripherally by attenuating BD-like changes.

The findings suggest that this study can be utilized as strong evidence that SIRT-1 downregulation and serotonin evaluation can be used as a potential biomarker for the early identification of BD. The absence of gross pathology and immunohistology research on the area-specific molecular mechanistic effect of SNL is the major drawback of this study. As a result, more preclinical investigations on the knock-in and knock-out of the SIRT-1 gene are needed to understand the molecular mechanism better.

Abbreviations

SIRT-1 :Silent mating-type information regulation 2 homolog-1

NAD+ :Nicotinamide adenine dinucleotide

BD :Bipolar Disorder

IL-1β :Interleukin-1β

AD :Alzheimer disease

PD :Parkinson's disease

MS :Multiple sclerosis

NADH :Nicotinamide adenine dinucleotide hydrogen

p53 :Tumour proteins p53

FOXO1/3 :Fork head box protein O1/3

PGC-1 :Peroxisome proliferator-activated gamma co-activator-1

NF-kB :Nuclear factor kappa light chain enhancer of activated B-cells

Na+K+-ATPase :Sodium and potassium-activated adenosine triphosphatase

1. **HT** :Serotonin

ALS :Amyotrophic lateral sclerosis

TNF-α :Tumour necrosis factor-alpha

AP-1 :Activator protein-1

ROS :Reactive oxygen species

RNS :Reactive nitrogen species

BDNF :Brain-derived neurotrophic factor

ATP :Adenosine triphosphate

BAX :Bcl-2-associated X protein

ERK1/2 :Extracellular signaling-regulated protein kinases 1 &2

CVS :Chronic variable stress

AchE :Acetylcholinesterase

CSF :Cerebrospinal fluid

FST : Forced Swim test

GSH :Glutathione

HPLC :High performance liquid chromatography

LDH :Lactate dehydrogenase

MDA :Malondialdehyde

v/v :volume/volume

SNL :solanesol

ICV :Intracerebroventricular

OUA :Ouabain

CoQ10 :Coenzyme Q10

Li :Lithium

ETC :Electron transport chain

HD :Huntington disease

ALS :Amyotrophic lateral sclerosis

ICH :Intracerebral haemorrhage

IAEC :Institutional AnimalEthics Committee

BAPEX :Bangladesh Petroleum Exploration and Production

SEM :Standard error of the mean

ANOVA :Analysis of variance

MDA :malondialdehyde

SOD :superoxide dismutase

LDH :lactate dehydrogenase

OFT :Open field test

Ca2+ :Calcium

Ach :Acetylcholine

FST :Force swim test

IP :Intraperitoneal

ELISA :Enzyme-linked immunoassay

SDH :Succinatedehydrogenase

PO :Per oral

OPA/β-ME :O-phthalaldehyde/β-mercaptoethanol

Declarations

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Author contributions

The authors declare that all data were generated in-house and that no paper mill was used. **Bidisha Rajkhowa (BR) contributed t**hesis research work, Performed experimental animal studies, Compilation of statistical research data; **Sidharth Mehan (SM) contributed** original research hypothesis, guide, and compilation of all manuscript data

Ethical approval

All applicable institutional guidelines for the care and use of animals were followed.

Consent to participate

Not applicable

Consent to publish

Not applicable

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Competing Interests

“The authors declare no conflict of interest.” “The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results”.

Availability of data and materials

All data generated or analyzed during this study are included in this article. There are no separate or additional files.

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Figures

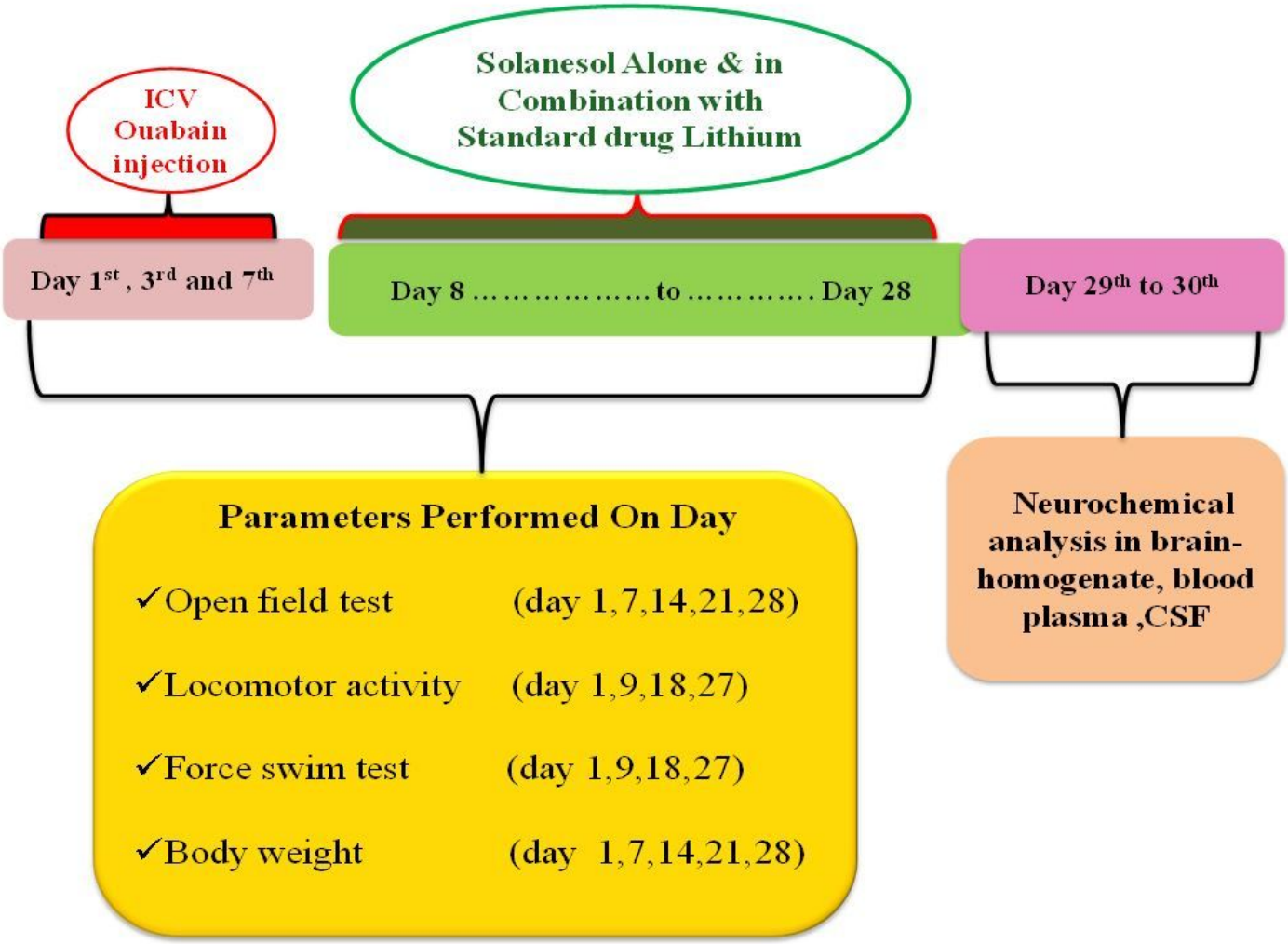

Figure 1

Experimental protocol schedule (Behavioral & Biochemical estimations)

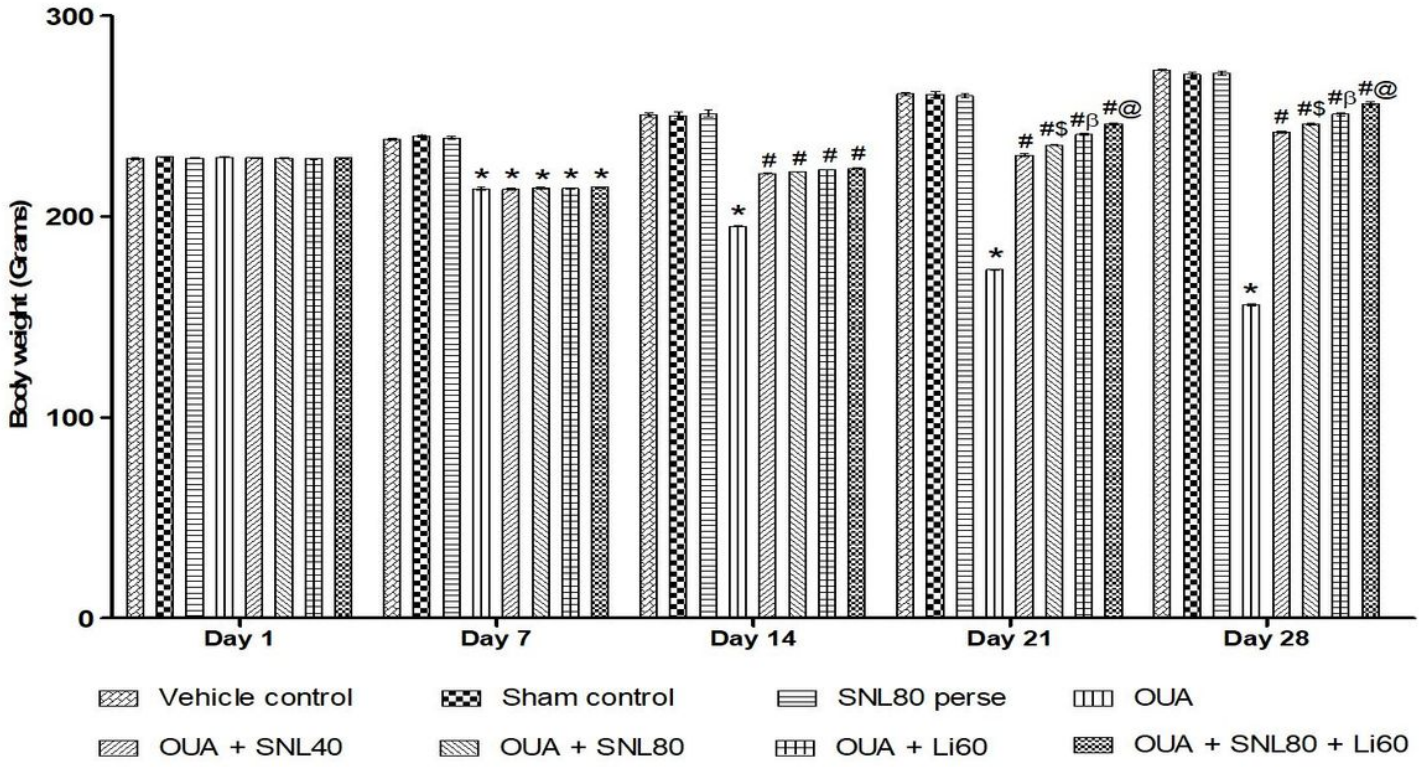

Figure 2

Neuroprotective potential of solanesol on body weight in ouabain-induced bipolar disorder rats Statistical analysis followed by two-way ANOVA (post-hoc Bonferroni's test).Values expressed as mean±SEM(n=6 rats per group). * p<0.001 v/s vehicle control, sham control and SNL80 perse; # p<0.001 v/s OUA; #$ p<0.001 v/s OUA + SNL40; #β p<0.001 v/s OUA + SNL40 and OUA + SNL80; #@ OUA + Li60

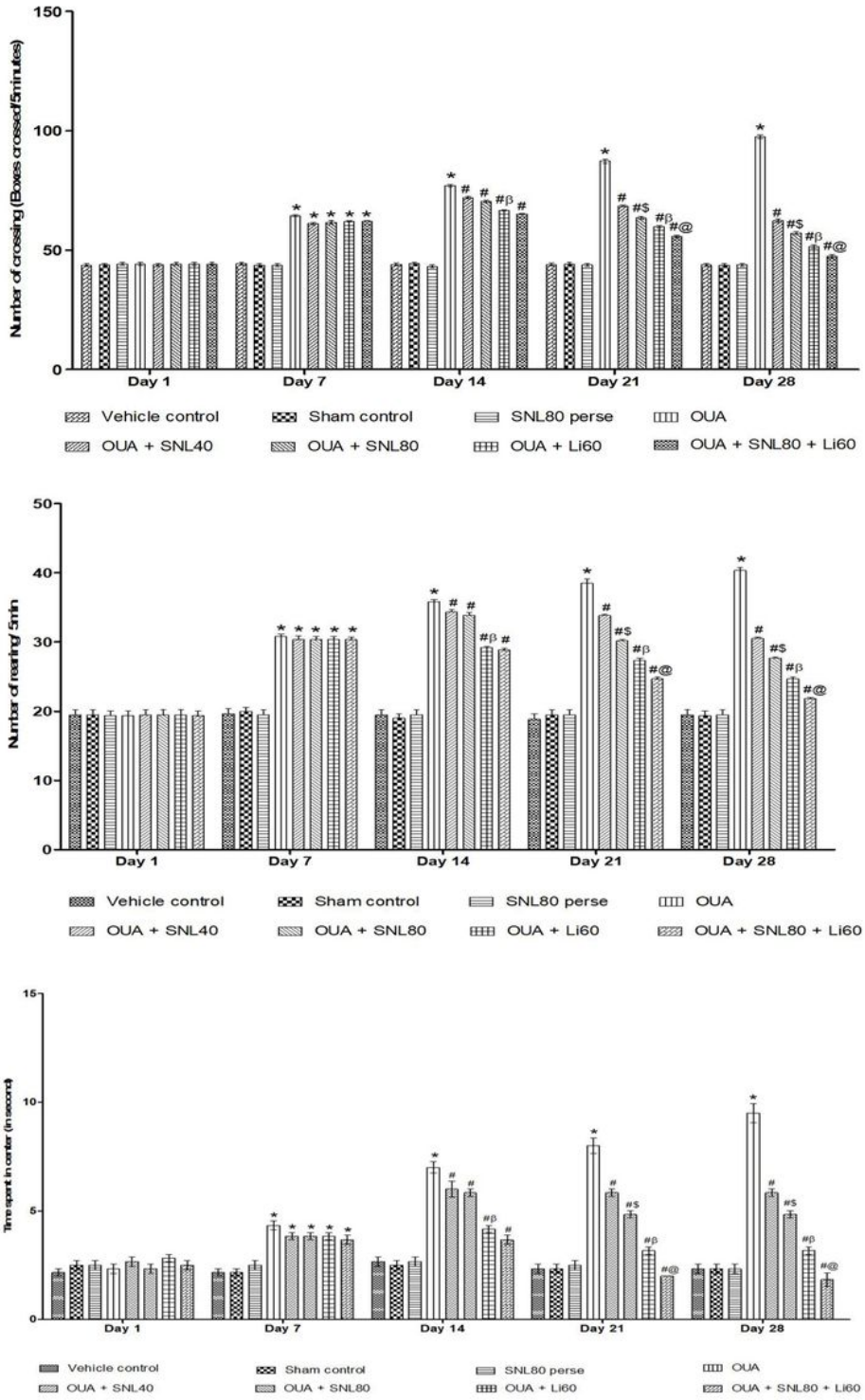

Figure 3

a: Neuroprotective potential of solanesol on Number of crossing in OUA induced bipolar disorder rats Statistical analysis followed by two-way ANOVA (post-hoc Bonferroni's test).Values expressed as mean±SEM(n=6 rats per group). * p<0.001 v/s vehicle control, sham control and SNL80 perse; # p<0.001 v/s OUA; #$ p<0.001 v/s OUA + SNL40; #β p<0.001 v/s OUA + SNL40 and OUA + SNL80; #@ OUA + Li60.

 Number of rearing in OUA induced bipolar disorder rats

Statistical analysis followed by two-way ANOVA (post-hoc Bonferroni's test).Values expressed as mean±SEM(n=6 rats per group). * p<0.001 v/s vehicle control, sham control and SNL80 perse; # p<0.001 v/s OUA; #$ p<0.001 v/s OUA + SNL40; #β p<0.001 v/s OUA + SNL40 and OUA + SNL80; #@ OUA + Li60. c: Neuroprotective potential of solanesol on in OUA induced bipolar disorder rats Statistical analysis followed by two-way ANOVA (post-hoc Bonferroni's test).Values expressed as mean±SEM(n=6 rats per group). * p<0.001 v/s vehicle control, sham control and SNL80 perse; # p<0.001 v/s OUA; #$ p<0.001 v/s OUA + SNL40; #β p<0.001 v/s OUA + SNL40 and OUA + SNL80; #@ OUA + Li60.

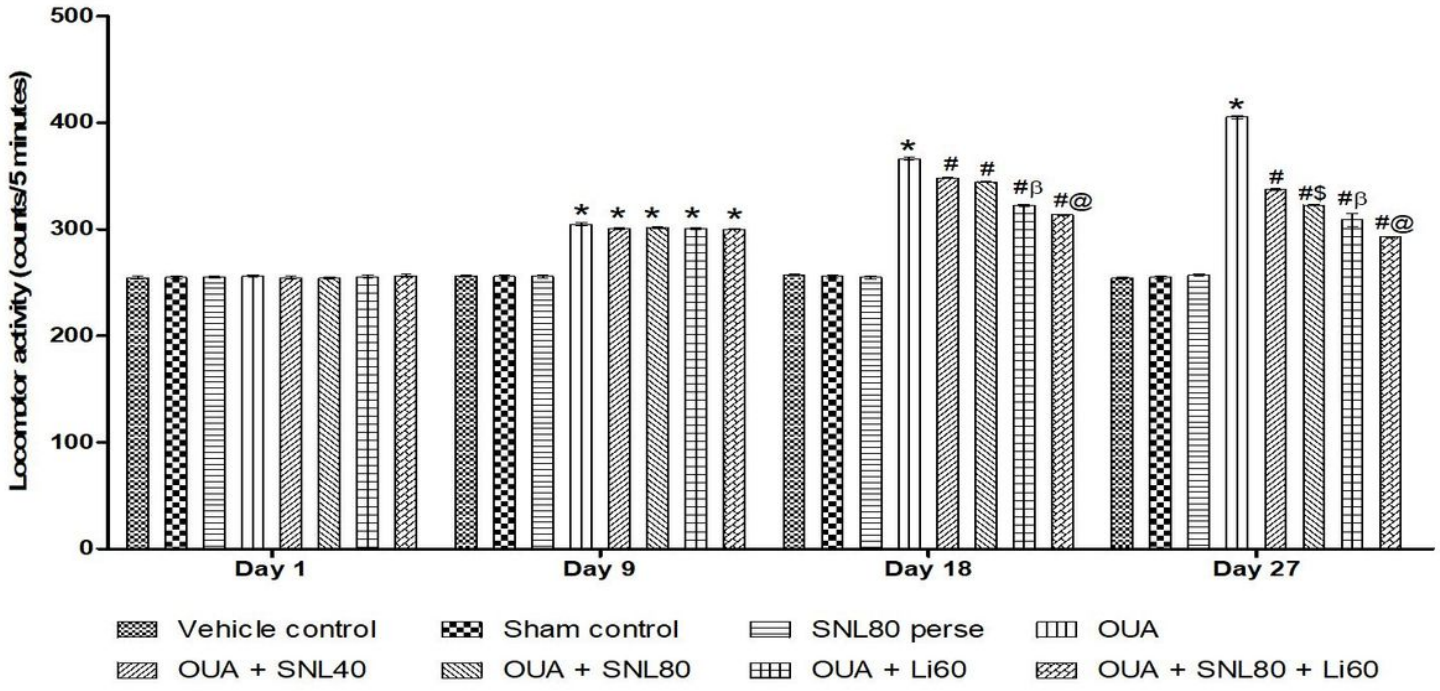

Figure 4

Neuroprotective potential of solanesol on marble buried behavior in OUA induced bipolar disorder rats Statistical analysis followed by two-way ANOVA (post-hoc Bonferroni's test). Values expressed as mean±SEM (n=6 rats per group). * p<0.001 v/s vehicle control, sham control and SNL80 perse; # p<0.001 v/s OUA; #$ p<0.001 v/s OUA + SNL40; #β p<0.001 v/s OUA + SNL40 and OUA + SNL80; #@p<0.001 v/s OUA + Li60

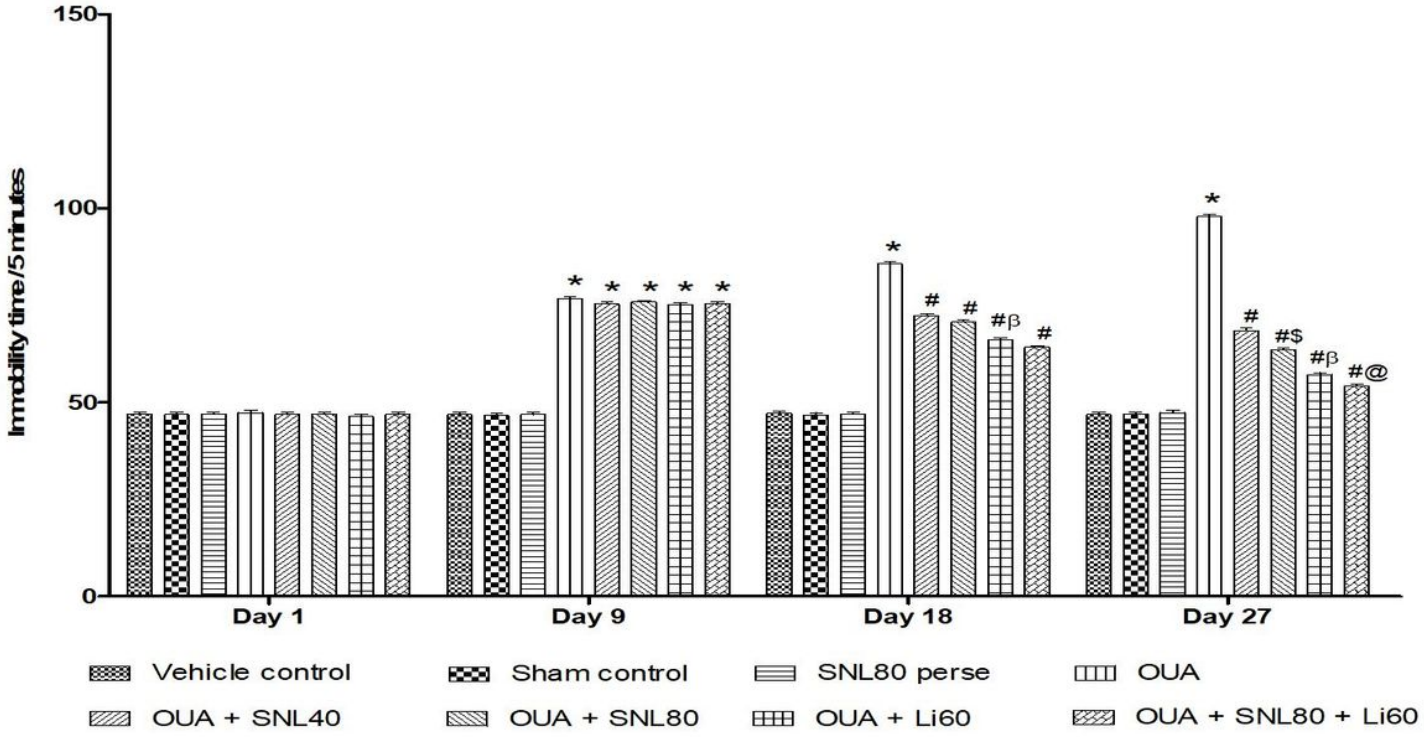

Figure 5

Neuroprotective potential of solanesol on immobility time in OUA-induced bipolar disorder rats Statistical analysis followed by two-way ANOVA (post-hoc Bonferroni's test).Values expressed as mean±SEM(n=6 rats per group). * p<0.001 v/s vehicle control, sham control and SNL80 perse; # p<0.001 v/s OUA; #$ p<0.001 v/s OUA + SNL40; #β p<0.001 v/s OUA + SNL40 and OUA + SNL80; #@ OUA + Li60

Supplementary Files

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