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ORIGINAL ARTICLE

Vinpocetine restores cognitive and motor functions in Traumatic brain injury challenged rats

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Abstract
Traumatic brain damage is common worldwide and the treatments are not well-defined. Vinpocetine is a synthetic derivative of the vinca alkaloid vincamine and is clinically being used for various brain disorders. Here in the current study, we have investigated the neuroprotective potential of vinpocetine against traumatic brain injury. TBI was induced by the Marmarou weight drop method in rats. Brain damage was evaluated using cognitive and motor functions and the alterations in biomolecules. Injured rats were treated with different doses of vinpocetine (2.5, 5, and 10 mg/kg) for 4 weeks. Traumatic brain injury in rats produced significant deterioration of cognition and motor functions, which was accompanied by increased oxidative stress and significant alterations in brain monoamine levels as compared with the sham control group (p < 0.05). Vinpocetine alleviated TBI-induced oxidative burden, altered neurochemistry, and improved the cognitive and motor functions as compared with that of the TBI control group (p < 0.05). The observed neuroprotective potential of vinpocetine may be due to the observed antioxidant potential and its ability to restore the levels of brain neurochemicals under stressed conditions. The outcomes of the current study may help the repositioning of vinpocetine for preventing or treating traumatic brain injuries.

Keywords Brain damage · Trauma · Vinpocetine · Cognition · Motor Functions · Brain Injury

Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability among youths in industrialized societies that imposes a substantial social and economic burden on the community (Humphreys et al. 2013) and is prevalent in both low and high-income countries and affects people of all ages. It has been predicted to overcome many other diseases as a cause of death and disability by 2030 (Martínez-Molina et al. 2022; Wido et al. 2022). Each year, more than 50 million new TBI cases occur worldwide, and over 90% are mild TBI (Lerouet et al. 2021). TBI may originate from road traffic incidents, falls, sports, military conflicts, and more recently terrorism (Lerouet et al. 2021). The level of injury varies but is broadly classified as primary and secondary injury. The primary injury which occurs during trauma includes direct mechanical damage to the neuronal and surrounding supportive cells and vascular structures while the secondary injury occurs within minutes after trauma.

Immediate care is required in case of primary injury like structural damage like bone cracks, tissue damage or vascular leakage etc. whereas secondary damage needs long-term treatments and a care such as cognitive impairment, movement disability and other psychological issues (Al-Sarraj 2016; Bagri et al. 2021). Eventually, co-morbidities including cognitive and motor impairments have been shown to contribute more than physical impairments to the overall disability after TBI. The secondary injury initiates progressive damage due to the activation of several signaling cascades, such as oxidative stress, apoptosis, inflammation, ischemia, mitochondrial dysfunction, neurotransmitter deficits, and excitotoxicity (Ghajar 2000; Greve and Zink 2009).

Secondary injuries are preventable, although the cause of functional disability may require hours or years to resolve. Agents targeting above said mechanisms may help to rescue patients with long-term disabilities like cognitive and motor dysfunction.

Vinpocetine is a synthetic derivative of vinca alkaloid vincamine, an alkaloid extracted from the periwinkle plant, Vinca minor Vinpocetine has been used clinically in many
Asian and European countries for the prevention and treatment of stroke, senile dementia, and memory disturbances (Zhang et al. 2018a). Vinpocetine has a wider action profile with different cellular targets. Cyclic nucleotide phosphodiesterase is among the first pharmacological target reported for vinpocetine (Park et al. 2003; Zhang et al. 2018b) In addition, vinpocetine also acts as a blocker for voltage-dependent Na+ channels, and more recently, vinpocetine was reported to be an inhibitor of IκB kinase (IKK) and produce anti-inflammatory action (Jeon et al. 2010; Zhang and Yan 2020). Vinpocetine has been demonstrated to be neuroprotective and helps in improving cognitive and motor functions in experimental models of neurodegenerative pathologies (Deshmukh et al. 2009; Sharma and Deshmukh 2015; Abdel-Salam et al. 2016; Ahmed et al. 2018; Shekarian et al. 2020; Han et al. 2021). Vinpocetine has been reported to possess antioxidant and anti-inflammatory potential and capable of improving mitochondrial biogenesis and cerebral glucose utilization etc. in various experimental models of brain disorders (Kiss and Karpati 1996; Nyakas et al. 2009; Dubey et al. 2020; Shekarian et al. 2020). As discussed before, traumatic brain injuries propagate damage through secondary injury, leading to cognitive and motor deficits and other neurological problems and vinpocetine may help to improve the therapeutic outcomes in addition to surgical care. Therefore, in the present study, we have investigated the therapeutic potential of vinpocetine in traumatic brain injury in rats.

Materials and methods

Experimental animals

Adult Sprague Dawley rats (either sex) weighing 200–300 gm were obtained from the Central Animal House facility at Maharaja Ranjit Singh Punjab Technical University, Bathinda, Punjab (India). The animals were housed in the polyacrylic cages under a controlled environment (room temperature 25 ± 2 °C and 60 ± 10% relative humidity) with 12 h light/dark reverse cycle. The animals were maintained on a commercial food diet (Ashirwad Industries, Mohali) in the form of dry pellets and water ad libitum. All the behavioral parameters were assessed between 9:00 am and 5:00 pm. The protocol of the study was duly approved by the Institutional Animal Ethics committee (IAEC) with approval no. MRSPTU/IAEC/2019/04 was carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the use and care of experimental animals.

Drugs and chemicals

Vinpocetine was received from M/S Covex Pharma, Spain as an ex-gratia sample. Vinpocetine was dissolved in saline. All the chemicals used in this study were of analytical grade. Solutions of the drugs were freshly prepared before use. The selected doses of vinpocetine are purely based on earlier reports (Rischke and Krieglstein 1991; Abdel-Salam et al. 2016; Ansari et al. 2019).

Induction of brain injury

Induction of brain injury is accompanied by the method described by (Marmarou et al. 1994) using a TBI device made by DoPST, MRSPTU, Bathinda. Animals were firstly anesthetized by intraperitoneally (i.p) administration of ketamine and xylazine in doses of 80 mg/kg and 5 mg/kg respectively. Around a 1 cm long incision was made on the scalp of the shaved head of the animal. The metallic disc was placed on the skull between bregma and lambda to prevent cranium injury. A freely falling cylindrical metallic weight of 250 gm was allowed to directly hit the head. The animal was laid on a form bed to prevent secondary body injury. The incision was sutured and Neosporin was sprinkled and gentamicin was given to avoid surgical infection (5 mg/kg i.p). After 1 h, sweetened milk was given to animals, and proper beddings and care was done (Fig. 1 and 2).

The animals were kept under rehabilitation for 14 days after TBI. Animals were regularly observed. Drug treatment was started on the same day of injury after 4–5 h and continued till the 28th day.

Experimental group and treatment schedule

Total 30 animals were used and were divided into five groups having six animals per group named as Sham
control, TBI Disease Control, Vin (2.5 mg/kg p.o), Vin (5 mg/kg p.o) and Vin (10 mg/kg p.o) and the detailed experimental protocol design is depicted as following:

Experiment design: Fig. 3

**Parameters assessed**

**Measurement of body weight**

The body weight was measured before induction of the injury and on the last day before sacrifice. The percentage change in the body weight was calculated as follows:

\[
\text{Change in body weight} = \left( \frac{\text{Body weight of 1st day} - \text{Body weight of last day}}{\text{Body weight of 1st day}} \right) \times 100
\]

**Behavioral parameters**

**Rotarod**

The aim of the rotarod is to test the motor coordination and balance of the body in case of rodents. This requires the animal to balance on the rotating rod and their latency of falling is meant as the endpoint and recorded. The rotarod (IMCORP, Ambala, India) is an automated apparatus with...
a 3 cm diameter “grooved rod,” speed controls, and a lever that triggers the timer to stop once the rat falls from the rod.

The animals were firstly trained for 3 days prior to starting the protocol. On the first day of the protocol before inducing injury the fall-off time was noted as basal readings. After 1st day on 14th as well as on the 28th-day readings were again recorded.

**Open field test (OFT) (locomotor activity)**

The open field apparatus was used to assess or evaluate spontaneous locomotor activity. OFT is a square arena with having white wall and floor having dimensions (70 × 70 × 25). Each animal was singly placed in the center of the open field and allowed to free range over for 5 min. The parameters analyzed included active time, passive time, and the total number of crossing from one quadrant to another. The whole parameter was analyzed by the Maze master video tracking system. The OFT apparatus was cleaned with 10% ethanol between the each session to remove the odor left by another animal.

**Object recognition test (ORT)**

This parameter is used to access the recognizing memory of the animals. On 27th and 28th days, ORT is done. Rats were individually placed in an open field arena having dimension (70 × 70 × 25) for 5 min for habituation in the arena, 24 h prior to the test. On the test day before taking readings the animals in were placed in the arena for 5 min. In the acquisition phase, two identical objects (FA1 and FA2) were taken such that they were heavy enough for the animals to not move them or climb over them; these objects were placed symmetrically. After 24 h, one of the object was replaced by a novel one (NO) to explore the behavior again for 10 min. In the open field arena and all objects were thoroughly cleansed using 70% ethanol between sessions to preclude odor recognition. The time spent near the object, rearing, sniffing at a distance of around 2 cm or touching was recorded. Successful recognition was revealed by preferential exploration of novel objects; discrimination of visual novelty was assessed by preference index.

\[
\text{Discrimination index} = \frac{\text{Time spent near novel object} - \text{Time spent near old object}}{\text{Time spent near novel object} + \text{Time spent near old object}}
\]

**Dissection and homogenization**

Animals were sacrificed on the last day of protocol and samples of the brain were isolated. 10% (w/v) tissue homogenate was prepared by using 0.1 M phosphate buffer (pH 7.4) in a homogenizer. The homogenate was centrifuged at 10,000 g for 15 min. The supernatant was separated and used for various biochemical estimations.

**Estimation of oxidative stress marker and acetylcholinesterase level**

**Measurement of lipid peroxidation**

The extent of lipid peroxidation was determined quantitatively by performing the method as described by Wills, 1966. The amount of malondialdehyde (MDA) was measured by reaction with thiobarbituric acid at 532 nm using a spectrophotometer. The procedure can be referred in our other research paper (Deshmukh and Sharma 2013).

**Estimation of nitrite**

The presence of nitrite in the supernatant, which is an indicator of the production of NO was determined by a colorimetric assay using Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide, and 5% phosphoric acid). The concentration of nitrite was expressed as μ mol per mg protein (Green et al. 1982). The detailed procedure is illustrated by (Deshmukh et al. 2009).

**Estimation of reduced glutathione**

Reduced glutathione was estimated according to the method described by Ellman et al. 1961. The results were expressed as μ ml of reduced GSH per mg protein and the detailed procedure can be referred by (Sharma and Deshmukh 2015).

**Estimation of superoxide dismutase**

Superoxide dismutase activity was assayed according to the method of Kono et al. The final results obtained were expressed in units/mg of protein, where one unit of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 50% (Kono 1978). For the complete procedure please refer (Deshmukh et al. 2009).

**Estimation of protein**

The protein content was evaluated by using a Biuret reagent. The peptide group of proteins forms a purple complex with copper ions in an alkaline medium. The OD of the samples was measured at 540 nm illustrated in (Sharma et al. 2018).
Estimation of acetylcholinesterase (AChE) activity

Brain acetylcholinesterase was estimated using the method of Ellman et al. and the illustration of the procedure is referred to (Deshmukh et al. 2009). The absorbance was then noted at 412 nm using a spectrophotometer for 2 min thereafter and the change in absorbance per min was calculated.

Neurochemical estimation

Estimation of brain catecholamines

The levels of catecholamines in the brain were estimated using the method described by Patel et al. 2005; Jamwal and Kumar 2016. The levels of catecholamines (DA, serotonin, and NE) in the brain were estimated by high-performance liquid chromatography (HPLC) using an electrochemical detector (ECD). A Waters standard system consists of a high-pressure isocratic pump, a 20 μl manual sample injector valve, a C18 reversed-phase column, and an ECD was used in the study.

Preparation of mobile phase: sodium citrate buffer was prepared by dissolving 860 mg sodium dihydrogen phosphate and 325 mg sodium citrate in 440 ml of HPLC water, 25 mM ethylene diamine tetra acetic acid (EDTA) and 2 mM sulphonic acid solution were prepared separately by dissolving 2 mg of EDTA and 45 mg sulphonic acid in 220 ml of HPLC water each in different containers. All three solutions were allowed to sonicate for 10 min. All three solutions were mixed in a single container, again sonicated for 10 min and pH was adjusted at 4. The solution was filtered through a 0.2 μ filter. Then 120 ml of acetonitrile was added to the above solution and allowed for sonication for 10 min.

Procedure: the electrochemical conditions for the experiment were +0.75 V, with sensitivity ranging from 5 to 50 nA. Separation was carried out at a flow rate of 0.8 ml/min. The samples (20 μl) were injected manually. On the day of the experiment, the frozen brain samples were thawed and homogenized in a homogenizing solution containing 0.2 M perchloric acid. Then, the samples were centrifuged at 12,000 g for 5 min. The supernatant was filtered through 0.22 mm nylon filters before being injected into the HPLC sample injector. Data were recorded and analyzed. The concentrations of the neurotransmitters and their metabolites were calculated from the standard curve generated using a standard in the concentration range of 10–100 ng/ml. The values are expressed as a percentage of the normal control group (Patel et al. 2005; Jamwal and Kumar 2016).

Results

Effect of vinpocetine on body weight against weight drop induced TBI in rats

Weight drop on the head of the animal induces TBI due to which there is a significant decrease in the body weight of the animals which may be due to the increased hypermetabolic state, thus when compared, of course, TBI-treated rats showed significant (p < 0.05) change in body weight as compared to the sham control group. Treatment with vinpocetine dose-dependently (2.5, 5 and 5 mg/kg p.o) and significantly revert back the change in body weight as compared to the TBI group (Fig. 4).

Effect of vinpocetine on parameters of locomotor activity and memory

Effect of vinpocetine on rotarod activity against weight drop induced TBI in rats

On induction of the injury the locomotor activity declines due to the direct impact of the weight on the CNS which makes the brain lose its control of movement. Due to this TBI showed a significant (p < 0.05) decrease in rotarod activity as compared to the sham control group (Fig. 5). Treatment with vinpocetine (2.5, 5 and 5 mg/kg p.o) significantly (p < 0.05) prevented the impairment in rotarod activity as compared to TBI group on 15th and 28th day. Vinpocetine shows dose-dependent improvement in the fall-off time on rotarod apparatus on the 15th as well as 28th day. All three doses showed significant effects between them.

Effect of vinpocetine on active time in open field apparatus against weight drop induced TBI in rats

As the induction of the TBI causes a gradual decrease in the locomotor activity due to the impact of the weight on the brain which causes the brain to abolish its control on the movement. The spontaneous active time was estimated by open field on days 1st, 15th, and 28th. On the 15th day, traumatized rats showed a significant decrease in active time in the open field as compared to sham control, while treatment significantly and dose-dependently increase the active time (Fig. 6a). The highest dose significantly elevates the active time spent by animals as compared to the lowermost and intermediate dose whereas, no significant difference was observed with 2.5 and 5 mg/kg doses of vinpocetine.

With the weight drop on the head, the movement declines and the animal prefer to stay in the passive or nonmoving stage as compared with the sham group. The passive time
was observed by open field on the 1st, 15th, and 28th day of the protocol. On the 15th and 28th days, the TBI group significantly enhanced the passive time spent by the animal as compared to the sham control group while on treatment with vinpocetine dose-dependently and significantly attenuates the passive time spent in the arena as compared to the TBI group (Fig. 6b). The highest dose significantly attenuates the passive time spent by animals as compared to the lowermost and intermediate dose whereas, no significant difference was observed with 2.5 and 5 mg/kg doses of vinpocetine.

Similarly, the zone entries decline in the case of the traumatized animals, the zone entries i.e. the total number of entries in the different zones were estimated by OFT on days 1st, 15th, and 28th. On the 15th and 28th days, the TBI group showed a significant effect ($p < 0.05$) with the sham group (Fig. 6c).

On treatment with vinpocetine dose-dependently and significantly ($p < 0.05$) increase the no of crossings as compared to the TBI group. Treatment with the highest dose i.e. Vin10 mg/kg found to be highly effective in treating the locomotion disability associated with TBI. The highest dose...
significantly elevates the zone entries of the animals as compared to the lowermost and intermediate dose whereas, no significant difference was observed with 2.5 and 5 mg/kg doses of vinpocetine.

The trajectory pathway followed by the animals in OFT was tracked by the Maze master video tracking system and depicted in Fig. 6d.

**Effect of vinpocetine on novel object recognition task against weight drop induced TBI in rats**

As the weight drop on the head causes TBI which may affect the normal physiological functioning of the hypothalamus as well as cortex which attenuates memory and cognitive abilities. On day 27th followed by TBI, the initial trial (T1) of the ORT was performed. Both the object was
similar to FA1 and FA2, all the rats took almost similar time except the TBI group for object exploration depicted in Fig. 7a.

Whereas on day 28th, when animals were exposed with the final trial (T2) with familiar (already exposed object FA1) and novel object (new object) showed significant discrimination (Fig. 7b). TBI control group showed a statistically significant deficit in their discriminatory index as compared to the sham control group \((p < 0.05)\) as traumatized rats were unable to discriminate between familiar and novel objects may be due to abnormal functioning of the hypothalamus. Whereas, vinpocetine treatment produced significant improvement in discriminating ability between familiar and novel objects as vinpocetine treatment show significant improvement in the discriminatory index \((p < 0.05)\) as compared to the TBI control group by possible attenuating the neuronal signaling and functioning of the hypothalamus and other brain parts. Vinpocetine-treated rats spent more time in exploring the Novel object \((p < 0.05)\). (Fig. 7c).
Fig. 7  

a Effect of vinpocetine on exploration time in NOR/ORT in TBI rats. Data analyzed by two-way repeated measures ANOVA followed by Bonferroni’s multiple comparison.  
b Effect of Vinpocetine on exploration time of FA (familiar) and NO (novel) objects in NOR/ORT in TBI rats. Data analyzed by two-way repeated measures ANOVA followed by Bonferroni’s multiple comparison.  
c Effect of vinpocetine on discrimination index in NOR/ORT in TBI rats. Data analyzed by one-way ANOVA followed by Bonferroni’s multiple comparison. Values are expressed as Mean ± SD.  
$^a p<0.05$ versus Sham control,  
$^@ p<0.05$ versus TBI control,  
$^\$ p<0.05$ versus Vin (2.5),  
$^* p<0.05$ versus Vin (5)
Effect of vinpocetine on lipid peroxidation (LPO) against weight drop induced TBI in rats

Weight drop on the head of the animals causes TBI, which was evidenced by the elevated level of oxidative stress markers in animals and reduced systemic antioxidant production. The brain MDA levels were estimated at the end of the protocol i.e. 28th day. TBI-induced rats (TBI control group) produced an elevation in lipid peroxidation as indicated by the significant rise in the MDA level ($p < 0.05$), indicating oxidative stress, which was treated with PDE 1 inhibitor-vinpocetine at the doses of 2.5, 5 and 10 mg/kg significantly attenuates TBI induced elevation in MDA level. Figure 8 indicates its antioxidant potential ($p < 0.05$). The highest dose i.e. 10 mg/kg showed a significant effect with lower and intermediate doses, proving its highest efficiency.

Effect of vinpocetine on reduced glutathione (GSH) levels against weight-drop-induced TBI in rats

Dropping the weight on the head of the rat causes an elevation in the ROS production and a decline in the systemic production of antioxidants including GSH, catalase etc.; thus, the state of oxidative stress arises. The TBI group showed a decrease in GSH levels as compared with the sham

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**Fig. 8** Effect of Vinpocetine on lipid peroxidation (LPO) levels in TBI rats. The level of MDA was significantly decreased by vinpocetine in comparison to traumatized rats. Data analyzed by two-way repeated measures ANOVA followed by Bonferroni’s multiple comparison. Values are expressed as Mean ± SD. $^#p < 0.05$ versus Sham control, $^@p < 0.05$ versus TBI control, $^\text{\$}@p < 0.05$ versus Vin (2.5), $^{*}p < 0.05$ versus Vin (5)

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**Fig. 9** Effect of vinpocetine on glutathione (GSH) in TBI rats. The level of GSH was significantly increased by vinpocetine in comparison to traumatized rats. Data analyzed by two-way repeated measures ANOVA followed by Bonferroni’s multiple comparison. Values are expressed as Mean ± SD. $^#p < 0.05$ versus Sham control, $^@p < 0.05$ versus TBI control, $^\text{\$}@p < 0.05$ versus Vin (2.5), $^{*}p < 0.05$ versus Vin(5)
group. Vinpocetine treatment dose-dependently and significantly elevates the GSH levels as compared with the head trauma control group. The highest dose showed a maximum response and showed a significant effect in comparison to the lower and intermediate doses (Fig. 9).

**Effect of vinpocetine on SOD levels against weight drop induced TBI in rats**

SOD has been found to play an important role in the antioxidant system of the body as it is known to detoxify reactive oxygen species under normal as well as impaired conditions. A significant reduction was seen in SOD levels in TBI rats as compared to the sham control group. Vinpocetine dose-dependently showed a significant increase in SOD levels as compared to the TBI group. Vin (10) showed maximal activity among all three dose regimens and showed significant effect as compared to the lowermost and intermediate doses. However, an intermediate and lowermost dose of vinpocetine is not significant to each other (Fig. 10).

**Effect of vinpocetine on brain catalase activity against weight drop induced TBI in rats**

Reactive oxygen species generated after brain trauma trigger a cascade of events resulting in neuronal death. Despite numerous defenses, the brain is vulnerable to oxidative damage, mainly because of its high level of ROS and decrease in antioxidant levels i.e. catalase, GSH, SOD etc. Thus, TBI-induced animals significantly decrease the activity of catalase in the brain in comparison to sham control animals ($p < 0.05$). Administration of vinpocetine dose-dependently and significantly restored the reduced activity of catalase in the brain in comparison to observed in TBI control animals. Vinpocetine treatment on the highest dose i.e. 10 mg/kg showed a significant effect in elevating the brain catalase level with that of intermediate as well as the lower dose, however, lower and intermediate doses were not found to be significant with each other (Fig. 11).

**Effect of vinpocetine on nitrite levels against weight drop induced TBI in rats**

Weight drop on the head of rats caused TBI which was evidenced by the rapid increase in nitric oxide within minutes following TBI. This elevation in nitrite levels is closely linked to a reduction in cerebral blood flow. The TBI group showed a significant increase in nitrite levels as compared with the sham group. Treatment with vinpocetine significantly and dose-dependently attenuated the nitrite levels as compared with the head trauma control group. The highest dose i.e. 10 mg/kg showed a significant effect with lower and intermediate doses, proving its highest efficiency. However, intermediate and lowest doses did not show any significant effect on each other (Fig. 12).
Effect of vinpocetine on brain AChE levels against weight drop induced TBI in rats

After dropping weight on the head of a rat, the level of ACh declines due to the elevated AChE level which increases the breaking of ACh into constituents affecting memory and cognitive impairment. TBI control group showed a significant increase in the levels of AChE enzyme as compared to the sham group \((p < 0.05)\). All three doses of vinpocetine treatment significantly attenuated the increased AChE activity as compared with TBI group animals \((p < 0.05)\). Treatment with the highest dose i.e. Vin10 mg/kg found to be highly effective in attenuating the AChE level. The highest dose significantly attenuated AChE in animals as compared to the lowermost and intermediate dose whereas, no significant difference was observed with 2.5 and 5 mg/kg doses of vinpocetine (Fig. 13).
Effect of vinpocetine in neurochemical estimation

Effect of vinpocetine in the estimation of catecholamines level

On induction of the injury, there is a decline in the level of catecholamines due to the increase in the level of its metabolism and receptor binding potential which affects mood, emotions, cognition etc. TBI control group showed a significant effect in attenuating the level of catecholamines (dopamine, serotonin as well as norepinephrine) level compared to the sham group ($p < 0.05$). All three doses of vinpocetine treatment significantly and dose-dependently restored the level of catecholamine compared with the TBI control group ($p < 0.05$). Vinpocetine 10 mg/kg significantly restores the catecholamine level compared to its lower and intermediate effect while no significant difference was observed with 2.5 and 5 mg/kg doses of vinpocetine i.e. almost found to be equi-effective (Fig. 14).
Discussion

The present study demonstrates the neuroprotective potential of vinpocetine against weight drop-induced traumatic brain injury (TBI) in rats. Herein the current study, weight drop-induced TBI caused a significant decrease in body weight and motor activity. Spontaneous locomotor activity and skilled movements including gripping ability were evaluated using open field and rotarod tests respectively, which are widely used to identify the functional ability of basal ganglia (Charrueau et al. 2009; Maurice et al. 2015). Clinically loss of skilled movements has been reported to occur in TBI patients like ideomotor apraxia is the loss of ability to correctly produce purposeful skilled actions (Heilman and Gonzalez Rothi 2012). Dopaminergic circuitry of the basal ganglia plays a major role in the control and execution of motor functions and damage to this area directly affects the movements (Ferrazzoli et al. 2018).

TBI rats were unable to perform skilled movements on rotating rods and showed decreased latency to fall and a similar decline was observed in the open field in the present study. The decline in body weight may be correlated with the associated hyper-metabolic response causing the elevation in resting energy expenditure which ultimately results in weight loss (Charrueau et al. 2009), whereas impaired motor activity indicates loss of basal ganglionic functions. In the present study, vinpocetine-treated TBI rats showed recovery in body weight as well as motor functions. Vinpocetine is reported to inhibit phosphodiesterase 1 enzyme as one of the major targets and improve cerebral cyclic nucleotide signaling (Kiss and Karpati 1996; Sharma et al. 2013; Dubey et al. 2020). On the other hand, vinpocetine has been reported to effectively target voltage-gated sodium channels and controls neuronal firing/ seizures (Abdelsayed and Sokolov 2013). As stated above vinpocetine-mediated improvement in cyclic nucleotide signaling has been linked to improved motor functions, mitochondrial biogenesis, and cerebral energy levels (Knott et al. 2017). Moreover, vinpocetine has also been reported to improve the levels of neurotrophic factors such as brain-derived nerve growth factor (BDNF) etc., which protect the neuronal cells under stressful conditions (Dutta et al. 2022). Indeed traumatic brain injury has been known to cause sudden disruptions in energy metabolism and makes the neurons hyper-excitable with loss of functional abilities (Benaroya 2020). Although it is difficult to ascertain the exact mechanism of vinpocetine, it would be safe to suggest that the modulation of cyclic nucleotide signals and inhibitory actions over sodium channels might have played a major role in the observed outcomes of the current study.

Clinically, mild to moderate cases of TBI patients have been observed with the cognitive deficit with or without motor disabilities. More than 75% of these injuries are considered mild traumatic brain injuries (mTBI), or concussions (Sosin et al. 1996) and neurocognitive dysfunctions including learning disabilities have been seen even in absence of any structural damage (Giza and Hovda 2001; Broglio and Puetz 2008). In the present study, to assess the neurocognitive functions we have used novel object recognition (NOR) task. The NOR task is particularly attractive because it requires no external motivation, reward, or punishment but a little training or habituation and it can be completed in a relatively short time (Silvers et al. 2007). The NOR paradigm gives the idea of spatial orientation of objects and is influenced by both hippocampal and cortical lesions (Clark et al. 2000; Buckmaster et al. 2004). It is widely accepted that in both the monkey and the rat brain, the perirhinal cortex plays an important role in object recognition memory (Aggleton et al. 2010).

In the present study, the traumatized rats did not perform well in the NOR task and the rats were unable to discriminate between novel and familiar objects indicating functional disability of the hippocampus and cortical brain regions. While vinpocetine treatment significantly attenuated TBI-induced cognitive deficit in the present study. As discussed before, vinpocetine has been reported to increase the levels of brain neurotrophic factors, which protects neurons under stressed conditions, rescue damage, and promote neurogenesis (Deshmukh et al. 2009; Ahmed et al. 2018). Thus the benefits observed following vinpocetine treatments may be due to its neuroprotective actions.

Cognitive and motor functions are the outcomes of neuronal communication through the release of neurotransmitters (Deshmukh et al. 2009; Deshmukh and Sharma 2013). Communication between neurons is an elegant process that facilitates the transmission of electro-chemical signals at synaptic junctions in the brain (SYNAPTIC 2012; Pereda 2014; Kaur et al. 2019). Insufficient release and imbalance in the levels of neurotransmitters at synaptic junctions have been well-documented to affect cognitive and motor functions (Westfall and Westfall 2011). Here in the current investigation, to understand probable reasons for cognitive and motor dysfunction, we have determined the levels of various neurotransmitters in the whole brain.

Neurotransmitters such as acetylcholine, serotonin, noradrenaline, and dopamine have been known to play a major role in cognitive and motor functions. For acetylcholine levels, we have evaluated acetyleholinesterase activity. In the current study, TBI produced a significant increase in AChE activity and a decline in monoamine levels in the rat brain. This may the reason for the observed deficit in cognitive and motor functions. Indeed, a similar decline in the levels of monoamines and elevated AChE activity have been reported earlier in traumatized rats’ brains (Ma et al. 2016; Kaur et al. 2018). On the other hand, vinpocetine treatment in TBI rats significantly restored brain monoamine levels and...
AChE activity, this can be well correlated with the improved cognitive and motor functions in the present study. In fact, vinpocetine has been used/tested in various experimental models and reported to possess nootropic potential (Kiss et al. 1982; Deshmukh et al. 2009; Jeon et al. 2010; Ahmed et al. 2018; Zhou et al. 2020; Han et al. 2021). Moreover, vinpocetine has been reported to improve the levels of cholinergic and monoaminergic transmitters and cognitive and motor functions in experimental models of neurodegenerative pathologies (Deshmukh et al. 2009; Abdel-Salam et al. 2016; Shekarian et al. 2020).

As discussed before, the deleterious pathways including neuroinflammation, oxidative stress etc. have been reported to contribute TBI related co-morbidities (Kochanek et al. 2006; Dixon 2017). Oxidative stress in particular may be an immediate step to disturbing the physiological processes. Indeed elevation in oxidative burden has been reported to occur in traumatized rat brains (Awasthi et al. 1997; Wang et al. 2019) as well as in humans (Halstrom et al. 2017). In the current study, a significant increase in oxidative stress was observed and is evidenced by increased levels of malondialdehyde (MDA) and nitrite levels, and a decline in glutathione (GSH), superoxide dismutase (SOD), and catalase were observed in traumatized rat brains. MDA is a well-known secondary product of lipid peroxide in myelin, glial and neural membranes and is formed from the breakdown of polyunsaturated fatty acids, serves as a convenient way for determining the extent of lipid peroxidation (Hall and Bosken 2009). The brain is particularly vulnerable to oxidative stress because of its high rate of oxygen consumption. Endogenous antioxidant enzymes such as GSH, SOD, and catalase, play an important role in safeguarding the body. However, the decline in antioxidant enzyme activities can contribute to an elevation in oxidative burden (Ozdemir et al. 2005). Herein the current study, we observed a significant increase in oxidative burden and a decline in antioxidant defense systems in traumatized rat brains. On the contrary vinpocetine treatment significantly altered oxidative stress and restored the antioxidant defense mechanisms and the observed effects are in line with earlier reports (Deshmukh et al. 2009; Deshmukh and Sharma 2013; Zhang and Yang 2015; Lourenco-Gonzalez et al. 2019; Shekarian et al. 2020).

Vinpocetine has been reported to improve mitochondrial biogenesis, having free radical scavenging and antioxidant properties (Abu-Elfotuh et al. 2022).

From the observed outcomes of the current study, it would be safe to conclude that vinpocetine by restoring the brain neurotransmitter levels and its antioxidant activity may be able to improve cognitive and motor functions in traumatized rats. Although vinpocetine was tested in various model systems for its neuroprotective potential, more studies are required in line to justify its clinical use in TBI patients. Nonetheless, the outcomes of the present study clearly indicate that vinpocetine could be useful in the management of co-morbidities associated with TBI patients.

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**Declarations**

**Competing interests**  The authors have not disclosed any competing interests.

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