Title: The Effect of Combination Therapy with Ethanol and Modafinil on Focal Cerebral Ischemia in Rats

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To appear in: Basic and Clinical Neuroscience

Received date: 2018/09/15
Revised date: 2019/02/16
Accepted date: 2019/04/13

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**Please cite this article as:**


DOI: http://dx.doi.org/10.32598/bcn.9.10.415
**Highlights**

- Combination therapy with modafinil and ethanol has a neuroprotective role.
- Modafinil and ethanol increased anxiety in Rats.
- Modafinil and ethanol improved locomotor activity and neurological functions.

**Plain Language Summary**

Brain injury, following the stroke, results from the interruption of blood flow to the brain and lack of oxygen in the damaged area. Stroke is the third leading cause of long-term disability and mortality in the world. People are annually affected by stroke, leading to a broad spectrum of physical and psychological disabilities.

There is currently no effective treatment for stroke, and the use of thrombolytic agents is the only available treatment for the management of stroke in patients. Nowadays, research has focused on neuroprotective agents against that prevent to worsen stroke. Ethanol is readily available, well tolerated and easy administration that the effect of which has been proven. The mortality and infarct volume after cerebral ischemia is reduced by light-to-moderate consumption of alcohol. Modafinil has antioxidant properties that protective effect on epilepsy and Parkinson's disease is confirmed. Modafinil, in contrast to ethanol, improves motor function and boosts locomotor activity that shows a stronger neuroprotective effect of modafinil. Also, the combination of ethanol with Modafinil enhanced the synergistic effect on motor function and locomotor activity. The anxiogenic effect was enhanced by the combination of ethanol and modafinil. The infarct size is the central phenomenon often evaluated in experimental studies, but nowadays, the assessment of behavioral functions is being applied to monitor the patients attacked by the stroke. For this reason, this study attempted to investigate the behavioral change of these compounds either in a single usage or in combination with each other.
ABSTRACT

Purpose: Ethanol is considered an effective agent in reducing brain stroke injury. In this study, we assessed the effects of modafinil along with ethanol as combination therapy on behavioral functions in Wistar rats.

Materials and methods: Right middle cerebral artery occlusion (MCAO) was performed. We divided the rats into nine groups (n=8 for each group). The following groups in this study were as i) MCAO control group (ischemia without treatment), ii) vehicle group, iii) modafinil group which were randomly subdivided into three groups received the different doses of modafinil (10, 30, and 100 mg/kg) for 7 days before MCAO, iv) ethanol group received 1.5g/kg ethanol simultaneously with reperfusion v) modafinil + ethanol group that were further subdivided into three groups received modafinil with different doses (10, 30, 100 mg/kg) 7 days before MCAO and received ethanol simultaneously with reperfusion. The motor behavior assessment was measured using the Garcia test after 24h, 48h, and 72h and the elevated body swing test was performed after 48h and 72h. The anxiety and locomotor activity were analyzed by the open-field test after 48h and 72h of post-ischemia.

Results: The results showed that neurological deficit score, locomotor activity, and unexpected thigmotaxis (anxiety) in ethanol alone treatment, modafinil (in a dose-dependent manner), and ethanol+ modafinil treatment groups were significantly higher than the MCAO control group.

Conclusion: Our finding confirmed that modafinil (100 mg/kg) in combination with ethanol (1.5g/kg) is beneficial for the recovery of neurologic functions and locomotor activity before the induction of stroke.

Keywords: Middle Cerebral Artery, Ethanol, Neuroprotection, Locomotion, Anxiety, Modafinil
Introduction

Brain injury following stroke results from the interruption of blood flow to the brain and lack of oxygen in the damaged area (Ferdinand & Roffe, 2016). Tissue plasminogen activators (tPA) which are currently used to treat the acute ischemic stroke. Because of having the adverse side effect (Maleki, Aboutaleb, & Souri, 2018) and limited therapeutic efficacy, only a few percentages of patients benefit from these types of medications (Ryan et al., 2013). Numerous research spanning the past several decades demonstrates significant therapeutic advances in the development of the neuroprotective compounds (Beraki et al., 2013). Ethanol as neuroprotective agent possesses useful properties such as readily availability, well toleration and easy administration, as well as merely diffusion across the blood-brain barrier (BBB) (Cai, Stevenson, et al., 2016). Numerous studies have recently reported that light-to-moderate consumption of alcoholic beverages has neuroprotective effects after the ischemia (Cai, Stevenson, et al., 2016; Cai, Thibodeau, et al., 2016; Kochanski et al., 2013; McCarter et al., 2017).

The previous study reported that ethanol has anti-oxidative and anti-inflammatory potentials that are important in reducing ischemic brain damage and improvement in sensory/motor neurological defects(McCarter et al., 2017). Studies showed that ethanol (1.5 g/kg) improved the motor behavior 2 to 28 days after the stroke (Zhang et al., 2012) and reduced the neurological deficits by 48% at 24 h post-reperfusion (Ryan et al., 2013). Combination therapy of ethanol (1.0 g/kg), hypothermia, and Normobaric hyperoxia result in more significant reduction in neurological deficits that evaluated at 24 h after t-PA administration (Cai, Stevenson, et al., 2016; Cai, Thibodeau, et al., 2016)

Recent studies have demonstrated a surprising correlation between modafinil and neuroprotection(Han, Chen, Liu, & Zhu, 2017). This compound decreases the fatigue in stroke survivors(Bivard et al., 2017). It was reported that intraperitoneal administration of modafinil
is effective to improve the motor behavior and reduce the ischemic lesion caused by unilateral microinjection of endothelin-1 (ET-1) (Ueki et al., 1993). The infarct size is the primary phenomenon often evaluated in experimental studies, but nowadays, the assessment of behavioral functions are being applied to monitor the patients attacked by the stroke (Bargiotas, Krenz, Monyer, & Schwaninger, 2012). Numerous behavioral tests have been developed with the intention of examining how the stroke induction affects the sensorimotor, cognitive function, and the emotional status in animal models (Ingberg, Gudjonsdottir, Theodorsson, Theodorsson, & Strom, 2015). The current study aimed to assess the potential neuroprotective and combinatory effects of modafinil and ethanol on behavioral function in the stroke-induced animal model.

**Material and Methods**

**Animal**

In this study, a total of 72 adult male Wistar rats weighing 280–300 g were used. The animals were housed in nine groups (8 per cage) in room temperature (22 –24 °C, 45–50% humidity) and 12 h light–dark cycle with free access to food and water.

Rats were used according to the national institutes of health guide for the care and use of laboratory animals. This experimental stroke research was approved by the Ethics Committee of the Iran University of Medical Sciences (ethical code: ir.iums.rec 1395.9221313204).

**Experimental design**

The rats were randomly assigned to nine groups (n = 8) as follows:

Middle cerebral artery occlusion group (ischemia without treatment) abbreviated as MCAO, Vehicle group (called Veh), modafinil group that was further divided into three subgroups
which received doses of 10, 30, and 100 mg/kg (M10, M30, and M100, respectively) for 7 days before MCAO, ethanol group (called E) which received 1.5g/kg ethanol on reperfusion, and Modafinil+ethanol group which was further divided into three subgroups that received doses of 10, 30, and 100 mg/kg (E+M10, E+M30, and E+M100, respectively) for 7 days prior to MCAO and ethanol simultaneously with reperfusion. Open field, and elevated body swing tests were carried out after 48h and 72h, and Garcia test was done after 24h, 48h, and 72h after the ischemia in all groups of rats.

**MCAO model**

Rats were anesthetized by the intraperitoneal injection of 10% chloral hydrate (400 mg/kg). A midline incision in the neck was made under a surgical microscope (Olympus Sxz12) to expose right common carotid as well as the external and internal carotid arteries. To perform MCAO, a silicone coat filament (Doccol Corp., Sharon, MA, USA) was inserted through the right external carotid artery until it reached the anterior cerebral artery (Mokudai et al., 2000; Sicard & Fisher, 2009). After 60 minutes, the filament was removed from the internal carotid artery. Immediately after the removal of the obstruction, Ethanol (1.5g/kg) was injected and reperfusion was performed in E and E+M groups. Modafinil (Dipharma, Milan, Italia) was dissolved in dimethyl sulfoxide (DMSO)(Bezu, Shanmugasundaram, Lubec, & Korz, 2016) and then injected for seven days at 8:00 am every day, and the last injection was conducted 30 minutes before the induction of the ischemic model. The animals’ body temperature was monitored by means of a rectal thermometer (Kent Scientific Corporation, Connecticut, USA) and maintained at 37 C through a 220 V lamp which was located next to the animals.

**Cognitive and Behavioral Analysis**
**Garcia behavioral assessment**

The somatosensory and motor behavior indices were performed after the induction of ischemia in rats using Garcia's index. Briefly, the six measured items possessed a total score ranging from 3 to 18 in a way that better sensorimotor performance received a higher score. The six items included spontaneous activity, symmetrical movement of the limbs-tail suspension, forepaw extension, climbing the wall of a wire cage, body proprioception, and response to vibrissae touch that was measured in rat in 24,48,72 h after ischemia. Items 1–4 (i.e. spontaneous activity, symmetrical movements, symmetry of forelimbs, and climbing the wall of a wire cage) were intended to measure motor performance and items 5 and 6 (i.e. reaction to touch and response to vibrissae touch) sought to measure sensory function. The day in the horizontal axis and neural defect score in the vertical axis was set to a maximum of 18.

The area of under curve In the form of a trapezoid in each day was calculated in different groups. (Ghahari, Safari, Joghataei, Mehdizadeh, & Soleimani, 2014).

For each rat, the AUC is calculated as follows: \[ \text{AUC} = \frac{(\text{NDS}_1 + 2\text{NDS}_2 + \text{NDS}_3)}{2} \]

\[ \text{NDS}_1 = \text{neural defect score in day 1} \]

\[ \text{NDS}_2 = \text{neural defect score in day 2} \]

\[ \text{NDS}_3 = \text{neural defect score in day 3} \]

**Elevated body swing test**

To perform the analysis of the experimental stroke in rodents, elevated body swing test was conducted by the method of Borlongan and Sanberg. The rats were put in a transparent cage and allowed to habituate for 2 min and attain a neutral position (set as having all four paws on the floor). Then, the animals were held in the vertical axis, defined as no deviation of more than 10° to either side. A swing was determined whenever the animal moved its head out of the vertical axis to either right or left side. Healthy animals approximately swung equally to
either side implying no brain dysfunction. Animals with a unilateral cerebral lesion, e.g., ischemic stroke, are expected to present a dominant/biased swing direction (Ingberg et al., 2015).

Open field test
The open-field test is used for the measurement of locomotion and anxiety for the evaluation of the stress or drug response. Rats were placed in a box (80 cm² chamber, 20 cm high walls) and the floor is divided into equal squares (5 × 5 cm) by 1 cm wide lines. The animals were positioned somewhere in the box and the video recorded for a specified time. Data were analyzed using EthoVision XT software, using the following parameters: distance moved (distance traveled (cm) and the time spent peripheral and central zone(Walsh & Cummins, 1976).

Statistical analyses
All data were analyzed by SPSS software version 22. One-way ANOVA was used for comparison among different groups followed by post hoc test (Tukey) for Garcia, open field, and elevated body swing test. All data were expressed as mean ± SEM, and P <0.05 was considered statistically significant.

RESULTS

Garcia behavioral assessments

As described in somatosensory and motor test (Garcia's index), the area under the curve (AUC) was used for determination of the neurological deficit score in ischemic rats (Fig. 1). The
neurological deficit score of E group (15± 5) was significantly higher than the MCAO (p<0.001) and Veh (P=0.034) groups. The neurological deficit score of E+M100 (39.21± 80) group was significantly higher than E(15± 5), M10 (28.33± 2.02), E+M10 (19.5± 1.08) groups(p < 0.0001).

Elevated Body Swing Test

Elevated body swing test and Garcia test were used for the measurement of the neurological deficit (motor function) (Fig .2A-B). The proportion of the left-side swings at 48h after the ischemia(Fig.2A) in MCAO group (1± 0) did not differ from the Veh group (1± 0)(p > 0.05). However, the proportion of the left-side swings in E+M100 (0.62± 0.05) groups were significantly lower than E(0.94± 0.52), M10(0.97± 0.05), E+M10 (0.93± 0.5) groups(P<0.0001).The proportion of the left-side swings at 72 hours after the ischemia(Fig .2B) was not significant among the groups(P>0.05).

Open Field Test

The open field test was used to determine the locomotor activity by the total traveled distance(cm/5min) assessed in ischemic rats (Fig .3A,4A). The total traveled distance(cm/5min) (Fig .3A) by rats in E group (2508 ± 61.15) did not significantly differ from the MCAO(2263 ± 111.72) and Veh(2340 ± 199.44) groups at 48h after the ischemia(P > 0.05). However, the total traveled distance(cm/5min) in E+M100 (3921± 80) was significantly higher than E(2508 ± 61.15), M10(2850 ±81.50), E+M10( 3317± 171) groups.(P < 0.0001)

The total traveled distance(cm/5min) in E group (2603± 48.5) was significantly higher than the MCAO(2266 ± 111.84) (p<0.01) and Veh(2294 ± 140.76)(p<0.01) groups at 72h after the ischemia (Fig. 4A). However, the total traveled distance(cm/5min) in E+M100 (4021± 69)
group were significantly higher than E(2603± 48.5), M10(2970±43.92), E+M10( 3236± 100.40) groups.(P < 0.0001)

The time spent in the central zone was recorded to determine thigmotaxis at 48h and 72h after the ischemia (Fig. 3B,4B). The time spent in the central zone for E group(56.66 ± 2.51) was not significantly different from the MCAO(66.33± 1.52) and Veh(62.66 ± 2.08) groups (p>0.05) (Fig. 3B) at 48h after the ischemia. However, the time spent in the central zone in E+M100 ( 10.33 ± 1.52) was significantly lower than E(56.66 ± 2.51), M10(49.33±2.51), E+M10( 43.66± 3.05) groups (P < 0.0001).

The time spent in the central zone for the E group(51.33 ± 1.52) was significantly lower than the MCAO(56.66± 2.08) and Veh(57.33 ± 1.52) groups(p<0.05) at 72h after the ischemia (Fig. 4B). However, the time spent in the central zone in E+M100 ( 12± 1) group was significantly lower than the E(51.33 ± 1.52), M10(44.33 ± 1.52), E+M10 ( 44.66± 2.08) (P < 0.0001).

Discussion
Our study indicated that both single and combination of modafinil (in a dose-dependent manner) and ethanol increased the anxiety but improved neurological function and locomotor activity in ischemia-induced rats. Our previous study showed that both single and combination of modafinil (in a dose-dependent manner) and ethanol decreased apoptosis and increased neuroprotective effect after focal cerebral ischemia(Abbasi, Shabani, Mousavizadeh, Soleimani, & Mehdizadeh, 2019). Light to moderate alcohol consumption decreases the mortality and infarct volume after the cerebral ischemia(McCarter et al., 2017). Cai et al. reported that the administration of ethanol as a single (1g/kg intra-femoral injection) and as a combinatory compound with normobaric oxygen and hypothermia reduced the neurological
deficits (Cai, Stevenson, et al., 2016; Cai, Thibodeau, et al., 2016). Wang et al reported that a dose of 1.5 g/kg ethanol exerts a neuroprotective effect when administrated 4 h after the onset of ischemia (Zhang et al., 2012) and improved the neurological function up to 48% (Ryan et al., 2013) and motor function about 2-28 days after the induction of stroke in rats (Zhang et al., 2012). McCarter et al. reported that a period of 8-week pretreatment with red wine and ethanol (1.4 g/kg/day) as a gavage improved the neurological functionality and enhanced the focal cerebral ischemia when given as a gavage 24h before the induction of cerebral ischemia. In this report, we demonstrated that the single use of ethanol (1.5g/kg) when administrated intraperitoneal reduced the neurological defects, however; this value was much lower when compared with modafinil (in a dose-dependent manner). This finding was consistent with the previous studies, however; it has been shown that a single injection of ethanol does not suffice to improve the neurological function (Fig. 1). The neuroprotective effect of modafinil has been studied in animal models of neurodegenerative diseases (Bibani et al., 2012). Ueki et al. reported that the ischemic striatal injury induced by the microinjections of ET-1 in the rat neostriatum is counteracted by modafinil (10, 30, and 100 mg/kg i.p.) in a dose-dependent manner (Ueki et al., 1993). In the present study, the intraperitoneal administration of modafinil (10, 30, and 100 mg/kg) improved the neurological function in a dose-dependent manner that is in agreement with the previous studies. Notably, the combination therapy of modafinil (10, 30, and 100 mg/kg i.p) and ethanol (1.5g/kg) reduced the neurological defect suggesting that the neuroprotective effect of ethanol could be amplified when combined with modafinil. The effect of modafinil is more pronounced in the treatment groups when compared with the other groups after MCAO. It was confirmed that modafinil could improve the behavioral deficit and protect the neuron lesion on Parkinson’s disease (PD) models thereby the antioxidant activity (Ando et al., 2018).
The current form of Elevated body swing test (EBST) was modified by Borlongan and Sanberg as a test for asymmetrical motor behavior in an animal model of ischemic stroke (Borlongan, Cahill, & Sanberg, 1995). In this report, we observed entirely left side swing after 48h in the Veh and MCAO groups. However, the other treatment groups specifically E+M100 group showed similar results to the normal rats. The left side swing was decreased in all groups and approximately reached the baseline level (0.5) 72h after ischemia. Studies performed on elevated body swing test have inconsistent results. In a survey conducted by Ingberg et al. they showed that after middle cerebral artery occlusion, rats swung contralateral to the infarct day one post-MCAo, but ipsilateral day three post-MCAo in which the shift was unexpected. Another research carried out by Katsumata and colleagues, they reported that after the induction of cerebral ischemia in the right hemisphere of the brain in gerbils while the left-biased swings (contralateral swing) were observed in the day of ischemia-induction followed by ipsilateral side bias on the second day of post-ischemia (Strom, Ingberg, Theodorsson, & Theodorsson, 2013). Depending on the ischemic region the result of elevated body swing test would be different suggesting that the striatal damage results in ipsilateral swing phase but the cortical or combined damage would be resulting in contralateral swing phase in animals (Johnston, Dillon-Carter, Freed, & Borlongan, 2001) Ingberg et al. revealed that there was not a significant difference in groups of animals with respect to the proportion of the cortex infarction. According to these studies elevated body swing test may not be recommended as a reliable test for the evaluation of the motor asymmetry after MCA in rats (Ingberg et al., 2015).

In comparison to control mice treatment modafinil at the doses of 90-180 mg/kg induced hyper-locomotion (Raineri et al., 2011). The formation of edema and inflammation are the primary causations having a significant role in neuronal death and development of brain lesions after the stroke (Sharma, Westman, & Nyberg, 1998). Systemic inflammation often leads to a decrease in locomotor activity. Zager et al. reported that modafinil could prevent the increase
of lipopolysaccharide (LPS)-induced inflammation accompanied by the alterations in cells behavior (microglia), the number of infiltration of leukocytes, and rupture of the BBB (Zager et al., 2018). López-Arnau et al. reported that mephedrone increases the locomotor activity in rats when combined with ethanol (Lopez-Arnau, Buenrostro-Jauregui, Camarasa, Pubill, & Escubedo, 2018). In our study, in animals treated with ethanol (1.5 g/kg) the total traveled distance (locomotion activity at 48 and 72 h after ischemia) was increased that was lowered in comparison with modafinil. Modafinil (at the doses of 30 and 100 mg/kg) when it is combined with ethanol increased the total traveled distance suggesting modafinil can promote the locomotion activity. This finding was consistent with the study by Zager et al, López-Arnau et al., in which they showed that the single use of either ethanol or modafinil along with the combination of these agents could enhance the locomotor activity that may stem from inhibiting GABAergic interneurons that leads to the increase in burst firing of dopamine neurons in the nucleus accumbens (NAc) (Mitchell et al., 2012). The other possibilities for such effect include suppressing the post-ischemic expression of adhesion molecules and inflammatory mediators and neutrophil infiltration by ethanol and modafinil (McCarter et al., 2017; Zager et al., 2018).

Thigmotaxis is a natural defensive response in which rats prefer to spend most of their time near the walls and avoid the open center (Lamprea, Cardenas, Setem, & Morato, 2008). In open field test, ischemic stroke-induced mice tend to spend less time at the center of the arena (Vahid-Ansari, Lagace, & Albert, 2016). In this report, we demonstrated that the single use of ethanol (1.5 g/kg) could increase the anxiety, however; the degree of anxiety was lowered as compared to modafinil (in a dose-dependent manner). The combination of ethanol with modafinil also increased the anxiety at 48 and 72 h after the ischemia. Various type of anxiety-altering response modafinil might produce in humans and different animal models. It has been demonstrated that single doses of modafinil are not able to decrease anxiety responses in the
rhesus macaque monkey. However, it causes the anxiolytic response in marmoset monkeys (Callithrix jacchus) after a single oral dose of modafinil (between 50–225 mg/kg). One study showed that Modafinil did not increase panicogenic behaviors in male Swiss albino mice (Mus musculus). These studies indicate that the contradictory effect of modafinil on anxiety in different animal models (Johnson & Hamilton, 2017). Modafinil administration resulted in decreased GABA concentrations and increased concentrations of glutamate in medial preoptic area and posterior hypothalamus. Administration of GABAA receptor agonists and GABAA receptor antagonists into Hypothalamus of rats decreased and increased anxiety respectively. Modafinil changed the activity of frontal cortex and hippocampus that play important roles in anxiety (Randall, Shneerson, Plaha, & File, 2003). In addition, Modafinil increased 5-hydroxytryptamine (5-HT) release from frontal cortex and amygdala (Ferraro et al., 2002) that associated with increases in anxiety (Randall et al., 2003).

Studies reported that the anxiolytic and anxiogenic effect of ethanol is almost dose- and species-dependent. A lower dose of ethanol (1 g/kg) had no anxiolytics effects, whereas higher doses of ethanol (2.5 and 3 g/kg) were found to be sedative in adolescent rats (Sakharkar et al., 2014). Correspondingly, other studies have shown that ethanol at a dose of 1% v/v ethanol decreased the anxiety in zebrafish (Johnson & Hamilton, 2017). Besides, ethanol shows withdrawal symptoms include anxiety, insomnia, and autonomic hyperarousal (Krystal & Tabakoff, 2002). Anxiety and depressions are symptoms of withdrawal syndrome of ethanol. The Lateral Habenula (LHb) is part of dorsal posterior thalamus (epithalamus) adjacent to the third ventricle. LHb hyperexcitability is associated with anxiety-like phenotypes. Increase in LHb excitability in withdrawal ethanol is due to an increase in glutamatergic transmission and reduce in M-type potassium channel (Shah et al., 2017). Anxiogenic effect of ethanol (1.5g/kg) was evident in our study at 48 and 72 hours after the induction of ischemia that might emanate from ethanol withdrawal leading to increased LHb excitability.
In conclusion, our finding confirmed that modafinil (100mg/kg) in combination with ethanol (1.5g/kg) is beneficial for recovery of neurologic function and locomotor activity when administered before the induction of stroke. However, these agents have anxiogenic effects at different doses on animal models. Further studies are warranted to illuminate the precise mechanism of modafinil in stroke.

Ethical Considerations

Compliance with ethical guideline

Rats were used according to the national institutes of health guide for the care and use of laboratory animals. This experimental stroke research was approved by the Ethics Committee of the Iran University of Medical Sciences (ethical code: ir.iiums.rec 1395.9221313204).

Funding

This study was financially supported by a research grant from the Iran University of Medical Science (grant number:29298) for a Ph.D. student thesis.

Conflict of Interest

Authors declare that there is no conflict of interest in this study.

Acknowledgments

We thank Sobhan Darou Pharmaceutical company for providing the pure form of modafinil for us.


Fig. 1. The comparison of neurological deficit scores (AUC) in different groups. ‘a’ denotes \( p \leq 0.05 \) when compared to E group, ‘b’ denotes \( p \leq 0.05 \) when compared to E+M10 group, ‘c’ denotes \( p \leq 0.05 \) when compared to M10 group. AUC: area under curve, MCAO: middle cerebral artery occlusion, Veh: Vehicle, E: ethanol(1.5g/kg), M10: modafinil(10mg/kg), M30: modafinil(30mg/kg), M100: modafinil(100mg/kg), E+M10: ethanol(1.5g/kg)+modafinil(10mg/kg), E+M30: ethanol(1.5g/kg)+modafinil(30mg/kg), E+M100: ethanol(1.5g/kg)+modafinil(100mg/kg)

Fig. 2. (A) EBST result after 48h in different groups. (B) EBST result after 72h in different groups. ‘a’ denotes \( p \leq 0.05 \) when compared to E group, ‘b’ denotes \( p \leq 0.05 \) when compared to E+M10 group, ‘c’ denotes \( p \leq 0.05 \) when compared to M10 group. MCAO: middle cerebral artery occlusion, Veh: Vehicle, E: ethanol(1.5g/kg), M10: modafinil(10mg/kg), M30: modafinil(30mg/kg), M100: modafinil(100mg/kg), E+M10: ethanol(1.5g/kg)+modafinil(10mg/kg), E+M30: ethanol(1.5g/kg)+modafinil(30mg/kg), E+M100: ethanol(1.5g/kg)+modafinil(100mg/kg)

Fig. 3. The effects of various doses of modafinil and ethanol 48h after ischemia in open field test in different group. (A) total traveled distance(cm/5min). (B) Time spent in central zone(sec). ‘a’ denotes \( p \leq 0.05 \) when compared to E group, ‘b’ denotes \( p \leq 0.05 \) when compared to E+M10 group, ‘c’ denotes \( p \leq 0.05 \) when compared to M10 group. MCAO: middle cerebral artery
Fig. 4. The effects of various doses of modafinil and ethanol 72h after ischemia in open field test in different group. (A) Total traveled distance (cm/5 min).(B) Time spent in central zone (sec).

‘a’ denotes $p \leq 0.05$ when compared to E group, ‘b’ denotes $p \leq 0.05$ when compared to E+M10 group, ‘c’ denotes $p \leq 0.05$ when compared to M10 group.

MCAO: middle cerebral artery occlusion, Veh: Vehicle, E: ethanol(1.5g/kg), M10: modafinil(10mg/kg), M30: modafinil(30mg/kg), M100: modafinil(100mg/kg), E+M10: ethanol(1.5g/kg)+modafinil(10mg/kg), E+M30: ethanol(1.5g/kg)+modafinil(30mg/kg), E+M100: ethanol(1.5g/kg)+modafinil(100mg/kg)

Figure1:

Figure2:
Figure 3:

Figure 4: