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INVESTIGATING THE EFFECTS OF DIFFERENT LEVELS OF CANOLA OIL ON MOTOR AND HISTOLOGICAL DISORDERS CAUSED BY MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO) IN RATS

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ABSTRACT

Stroke is the third cause of mortality in developed or developing countries and one of the major causes of long-term disabilities. Every year, a large number of people in different societies develop some degrees of stroke and the consequent complications due to a variety of reasons. Cerebral ischemia is the acute nerve damage caused by impaired blood supply to some part of the brain that in the present study was created by middle cerebral artery occlusion (MCAO). In the present study, 100 male Wistar albino rats with weight range of 300-350 gr were divided into 5 groups; a control group, ischemia group, ischemia groups treated with 0.25, 0.5, and 0.75 ml/Kg canola oil. The present study was aimed at investigating the effect of canola oil on motor and histological disorders caused by MCAO among rats. The results of the study indicated that canola oil has a strong antioxidant effect and can reduce the rate of neuronal death and brain edema in the experimental model of focal cerebral ischemia by decreasing the production of free radicals and strengthening the cellular antioxidant system. It can also modify motor disorder caused by cerebral ischemia and reduce the volume of the ischemic area to a significant extent ($P<0.05$).

Key words: Canola Oil, Histological Disorders, Middle Cerebral Artery Occlusion (MCAO)

INTRODUCTION

Stroke is a syndrome characterized by an acute beginning of a neurologic deficit which at least continues 24 hours and is the sign of focal involvement of the central nervous system caused by circulatory disorders of the brain [Mahmoud Robati et al. 2003]. It is the third common cause of mortality and the most common debilitating neurological disorder among adults, which increases with age. Prevalence of stroke in recent decade has decreased, which is mainly attributed to improvement in and treatment of high blood pressure. However, among neurological diseases, stroke is placed first in regard with significance and rate of prevalence such that stroke is the mortality cause of 9.5% of the cases. More than 70% of the individuals with a stroke cannot do their activities and are disabled. More than 30% of them need others to help them do their daily errands, and only 20% can walk with a walking aid [Seifi et al. 2011].

Ischemia is defined as lack of blood flow in a part of the body caused by constriction or obstruction of arteries. Due to lack or absence of blood flow in this conditions, exact transfer of needed materials such as oxygen and glucose will be disrupted. On the other hand, removing waste from the tissue will also be interrupted, which can cause irreversible cellular damages or tissue death (infraction) depending on intensity and duration of ischemia [Malek, 2010]. Recent experimental research indicated that edema and brain inflammation and also neuronal damage that secondarily cause cerebral ischemia are the main causes of mortality and motor disorders followed by ischemia [Zargari 1997]. Most types of ischemia are caused by occlusion of one of the cerebral arteries and its branches. Nowadays, the model of Middle Cerebral Artery Occlusion (MCAO) is considered as a common model to study cerebral ischemia.

Canola refers to species of turnip belonging to wallflower family and brassicaceae from which canola oil is produced. These species include brassica napus and brassica rapa [Gupta & Briyal 2004, Malek, 2010]. Canola is an herbaceous plant with an erect branched stem whose heights varies between 50 and 150 cm. It has a straight and non-swollen root. Its flowers are yellow, and its inflorescence is like a long cluster. Canola fruit is pod-like and 5-10 cm. Each pod has two carpels. There are

15-40 seeds in every pod. The seeds are spherical in shape and in color yellow, dark brown, or black [Zargari 1997, Seifi et al. 2011]. The present study was conducted in order to investigate the effects of canola oil on cerebral damages by the model of cerebral ischemia in rats.

MATERIALS AND METHODS

In the present study, 100 male Wistar albino rats with weight range of 300-350 gr were investigated. They were bought from Razi Vaccine and Serum Research Institute, and before the study they were taken to and kept in the animal house of Science Faculty, Islamic Azad University, Mashhad. The animals were kept at a controlled temperature of 22±1˚C and the environment was 12 hours light and 12 hours dark. During their stay at the animal house, they had free access to water and food. To conduct the study, the rats were divided into the following 5 groups. All of the animals were retrained for 3 days to be able to settle in Traction Test device. Ischemia was created through MCAO. In this method, after the common carotid artery and its branches (internal and external carotids) were disconnected, nylon suture No. 0-3 with rounded tip was inserted into the internal carotid and slowly directed into the brain and the circle of Willis so it blocked the middle cerebral artery at the exit of the circle of Willis. After one hour, the suture was extracted and the surgery location was stitched.

1. Control group: In this group, the animals were gavaged with distilled water for 30 days, and at the end of the 30th day, they were anesthetized and the same surgery was carried out by MCAO method; however, no artery was blocked.
2. Ischemia group: In this group, the animals were gavaged with distilled water for 30 days, and at the end of the 30th day, they were anesthetized and the same surgery was carried out by MCAO method.
3. Ischemia group treated with 0.25 ml/Kg canola oil: In this group, the animals were gavaged with canola oil with dose of 0.25 ml/Kg for 30 days. At the end of the 30th day, they were anesthetized and the same surgery was carried out by MCAO method.
4. Ischemia group treated with 0.5 ml/Kg canola oil: In this group, the animals were gavaged with canola oil with dose of 0.5 ml/Kg for 30 days. At the end of the 30th day, they were anesthetized and the same surgery was carried out by MCAO method.
5. Ischemia group treated with 0.75 ml/Kg canola oil: In this group, the animals were

gavaged with canola oil with dose of 0.75 ml/Kg for 30 days. At the end of the 30th day, they were anesthetized and the same surgery was carried out by MCAO method.

2.1. Middle Cerebral Artery Occlusion (MCAO)

To create the model of stroke by the method of MCAO, mature male rats with a range weight of 300-350 were anesthetized using chloral hydrate at a dose of 350 milligrams per kilogram of body weight by intraperitoneal method. Under surgical operation, a gap was created in the middle line of the neck so that the common carotid artery appeared. Common carotid artery and its branches including external carotid artery (ECA) and internal carotid artery (ICA) were split and the common carotid artery was precisely separated from the adjacent nerve, and at the branching point of the internal and external carotid arteries it was closed permanently with a string. The external carotid was also separated and closed a little over the branching point of the common carotid artery. Afterwards, a 5-cm single-filament nylon suture 0-3 with a rounded tip with flame was directed into the common carotid lumen. In so doing and to prevent bleeding, the internal carotid was temporarily blocked with a curved clamp beneath the artery, and the common carotid artery was split using delicate scissors of ophthalmology, and after the suture was inserted, a tangent thread was fastened in order to prevent bleeding once the clamp was removed. Afterwards, the suture was directed into the internal carotid through the common carotid.

After the pterygopalatine artery (PPA) was identified, it was temporarily was blocked using a clamp in order to prevent bleeding. The nylon suture was directed into the internal carotid lumen until it reached the skull base. After about 20 millimeters, t was felt that the suture reached a barrier, which indicated that the suture had passed the position of the middle cerebral artery (MCA) and reached the anterior cerebral artery (ACA) that has a smaller diameter. At this point, the suture blocked the inside part of the luminal position of MCA. Therefore, all locations of blood flow from ICA and ACA and posterior cerebral artery (PCA) were blocked. One centimeter of the nylon suture was left out so that re-creating the blood flow could be possible. After 15 minutes, the blood flow of MCA was returned by pulling back the suture. The results of the experiment were figured out 24 to 72 hours after the experiment.

RESULTS AND DISCUSSION

Traction test, test of cerebral tissue, and measurement of the weight of ischemia brain were employed for all of the animals in the 5 groups in order to examine motor, neurological, and balance disorders.

3.1. Examining motor disorder

Traction test is a test in which a wire with a diameter of 2 mm and a length of 60 cm is placed horizontally at a height of 50 cm. The rat is forced to use its front paws to hang from the wire to prevent its fall. Afterwards, the times are measured. Healthy rats can hang for 60 seconds. This test was administered 3 times and the resulting means were reconsidered.

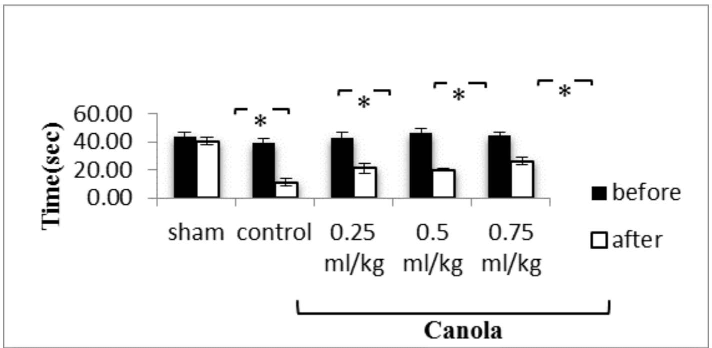

Fig 1: Comparison between endurance time in the test of arm traction before and after the MCAO surgery in all groups. * Means the significance of the results (P<0.05)

There were 6 rats in the test. The results were expressed in the form of Mean ±SEM using paired samples t-test. The endurance time obtained from the arm traction test in the ischemia control group and ischemia groups treated with 0.25, 0.5, and 0.75 ml/Kg canola oil significantly decreased after the MCAO surgery compared to before the study.

As is observed in Fig 1, the endurance time in the traction test in the control group and those treated with canola oil after the surgery compared to before the study reduced significantly ($P<0.05$). The Fig indicates that using canola oil has caused the endurance time after the test after the surgery to significantly increase ($P<0.05$).

According to this Fig, the after-surgery endurance time in the arm traction test in the control group compared to the healthy group had a significant reduction. Moreover, the control group compared to the ischemia groups treated with doses of 0.25, 0.5, and 0.75 ml/Kg canola oil experienced a significant decrease. There was no significant difference between the after-surgery endurance times in the arm traction test in the ischemia groups treated with doses of 0.25, 0.5, and 0.75 ml/Kg canola oil. However, compared to the healthy group, the group treated with doses of 0.25, 0.5, and 0.75 ml/Kg canola oil experienced a significant reduction.

3.2. Measuring the damage to cerebral tissue

Brain matrix is a frame the size of the rat brain. There are very delicate grooves with distances of 2 mm. using these grooves, 2-mm cross-sectional cuts can be prepared from the rat's brain. The matrix is typically utilized in microscopic examination of brain tissues without the tissue being fixed.

To measure the volume of the stroke, first the rats were killed with ether and beheaded, and then the samples were extracted and stored in saline 4 for 15 minutes. Afterwards, brain matrix was used to make 2-mm cuts. The cuts were stored for 15 minutes at temperature of 37˚C in 2% solution of triphenyltetrazolium chloride-2,3,5 (TTC). Then, they were filmed with a digital camera that was connected to a computer. After the photographs were transferred to the computer, the area of white and black parts, respectively as the damaged and healthy parts, was measured using Image Tools Software, the results of which are presented in Figure 2.

As indicated in Figure 2, there was no significant difference between the healthy group and the ischemia ones treated with doses of 0.25, 0.5, and 0.75 ml/Kg of canola oil. Moreover, in the ischemia group treated with 0.5 ml/Kg canola oil compared to the one treated with 0.25 ml/Kg canola oil, the damaged area has decreased. And, in the ischemia group treated with 0.75 ml/Kg canola oil compared to the ones treated with 0.25 and 0.5 ml/Kg canola oil, the damaged area has dropped.

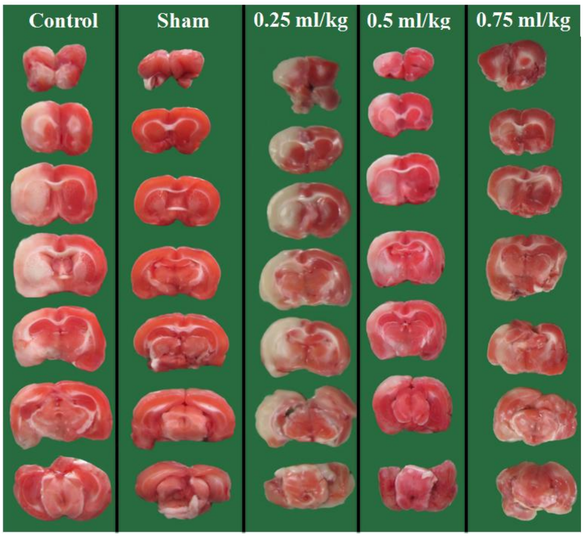

Fig. 2: Comparing the photographs taken from the brain tissue colored by the method of TTC 24 hours after reperfusion in all groups. The white area indicates the ischemia area and the red one shows the healthy part of the brain

3.3. Measuring the weight of the brain

To measure the weight of the ischemia brain, first the rats were killed and their skulls were removed and the brains were extracted, and after the wastes and the blood were removed, the brains were weighed using a digital scale. The results were expressed in the form of Mean ±SEM and through one way ANOVA test and are presented in Figs 3 and 4 below. According to these results, the after-surgery weight of the brain in the control group compared to the experimental group has experienced a significant rise; therefore, the surgery and creation of cerebral ischemia were successful. The after-surgery weight of the brain the ischemia groups treated with 0.75 and 0.5 ml/Kg canola oil compared to the one treated with .25 ml/Kg had a significant drop. However, the brain weight in the ischemia group treated with 0.75 ml/Kg canola oil compared to the one treated with 0.5 ml/Kg canola oil had no significant difference.

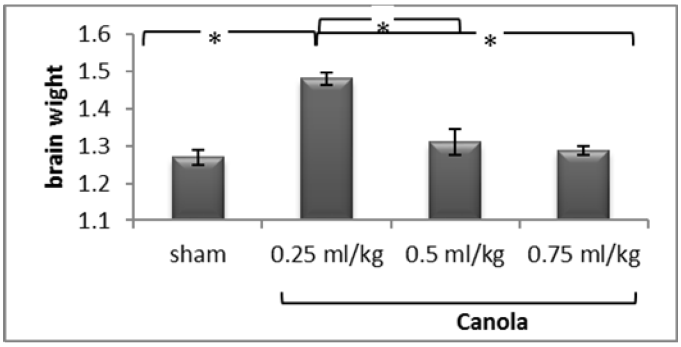

Fig 3. Comparing the after-surgery brain weight in the health group and the ischemia ones treated with 0.25, 0.5, and 0.75 ml/Kg canola oil. * Means the significance of the results (P<0.05)

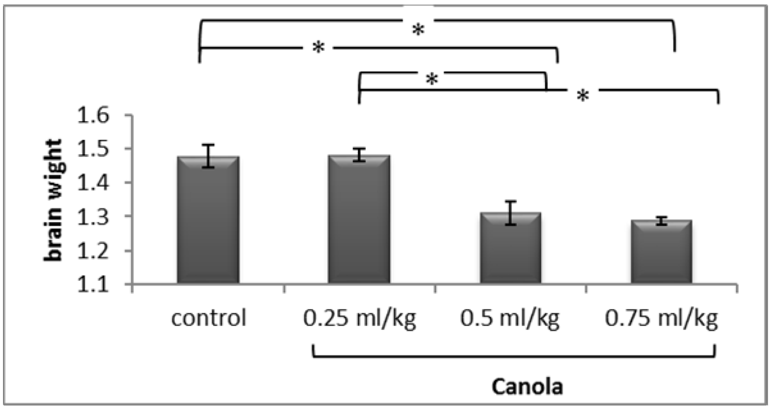

Fig 4. Comparing the after-surgery brain weight in the control group and the ischemia ones treated with 0.25, 0.5, and 0.75 ml/Kg canola oil. * Means the significance of the results (P<0.05)

CONCLUSION

Nowadays, it is ascertained that a reduction in the activity level of superoxide dismutase and glutathione peroxidase enzymes during focal cerebral ischemia may increase the sensitivity of nerve cells to damaging factors like free radicals and beginning of the planned cellular death; therefore, returning and maintaining the activity of cellular internal surface of these enzymes by motivating its synthesis increase may protect the cells against damage and death. Canola oil and its effective materials strengthen the antioxidant system and reduce the production of free radicals. They also reduce nerve death and cerebral edema in the experimental cerebral ischemia by increasing the activity level of antioxidant enzyme.

The results of the present study indicate that canola oil has a strong antioxidant effect and can reduce nerve death and cerebral edema in the experimental focal cerebral ischemia by increasing the activity level of antioxidant enzyme. It can also modify the motor disorders caused by cerebral ischemia and reduce the ischemia area to a significant extent.

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