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THE EFFECTS OF HYDRO ALCOHOLIC EXTRACT OF SALVIA RHYTIDEA ON TAC OF PLASMA AND STOMACH TISSUE EXTRACTS INDUCED GASTRIC STOMACH ISCHEMIA REPERFUSION IN RATS

MINA TADJALLI [*1], AMINEH DANESHI [1]

1- Department of Basic sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

*Corresponding Author : Mina Tadjalli , Department of Basic sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran , Email : m.tajali6@yahoo.com, Tel :+989171135339

ABSTRACT

Ischemia reperfusion injury as they could result in severe damages in tissues and organs and in clinical conditions such as cardiovascular problems, heart and brain stroke, trauma, shock, sepsis, and so reduce oxygen Decreased blood flow and tissue damage occurs.The damage caused by ischemia reperfusion Histomorphometrical study on rat stomach and the possible effects of alcoholic extract of salvia rhytidea salvia rhytidea on the changes.

Analysis method

histomorphometric study to assess the potential effects of gastric-alcoholic extract of Salvia rhytidea field and the changes were made.

Methods:In this experimental study 35 male Wistar rats weighing 300-350 g. Rats were randomly divided into 7 groups, each consisting of 5 mice as follows:

Group 1) negative control: sampling was done without ischemia.

Group 2) positive control: celiac artery ischemia in the stomach through close contact for 30 minutes and reperfusion for 30 or 60 minutes and blood sampling was performed after reperfusion.

Sham: manipulation celiac artery without ischemia-reperfusion by administering saline and sampling

The treatment group: was divided into four subgroups. The recipients received 2/3 mg/kg BW salvia rhytidea extract through gavage, respectively 72, 48, 24 and 0 hours before ischemia and sampling was performed after 24 hours.

Data were analyzed using statistical software SPSS-18 and comparison between treatments, analysis of variance (ANOVA) and Tukey's test was used for diagnosis.

Results

Salvia rhytidea extract antioxidant capacity compared with the sham and control group was significantly increased ($p < 0.05$). And, despite an increase in total antioxidant capacity in the other treatment groups that was not statistically significant, it can be said that increased salvia rhytidea extract on plasma TAC function is the time factor.

Conclusion

The results of this study showed that the total antioxidant capacity changes in tissue requires considerable time to obtain the extract. Salvia rhytidea extract only has a certain time period to affect tissue extract and deal with oxidative stress.

Keywords: Ischemia, reperfusion, stomach, salvia rhytidea.

INTRODUCTION

The most common medical cause of ischemic damage is often due to decreased blood flow in tissue specific vascular bed. Delivery ischemia reduces the substrate for glycolysis. In ischemic tissue cells after the existing substrates

Energy production through anaerobic stops (Robbins, 2004).

Reperfusion injury (restoration of blood flow) refers to tissue damage after ischemia and reperfusion more than 10 minutes in the treated tissue. More than ischemic reperfusion damage to the tissue was largely due to the production of free radicals during this phase (Aust, 1998; Robert, 1996).

Ischemia and reperfusion injury are a sequence of chemical events that can lead to false performance and cellular necrosis and is made of two parts: (1) direct damage: slight damage and ischemia occurs during this phase. (2) indirect damage: during reperfusion this causes tissue damage to occur and happens more than 10 minutes after the ischemic phase, blood flow is re-established and blood comes (Aust, 1998; Robert, 1996).

Ischemia followed by reperfusion IRI (Injury Ischemic Reperfusion) occurs (Veighey&Macallister, 2012), causing severe damages to tissues and organs such as cell

death. A common cause is an acute injury (Devarajan, 2006) that in situations like cardiovascular problems, stroke and heart attack ,trauma, shock, sepsis, vasoconstrictor drugs, drugs used in radiology, etc. that reduce Hypoperfusion and hypoxia (Sharfuddin&Molitoris, 2011; Veighey&Macallister, 2012).

Ischemic stroke is caused by blocked blood vessels, construction or functional damage in the stomach and the elderly, and this gradually returns to the main circuit and blood perfusion in elderly again after severe ischemia. Several mechanisms have been proposed to explain this phenomenon, ATP depletion and disruption of intracellular calcium homeostasis during ischemia are the reasons that led to the loss of live cells in elderly (John et al., 1978).

Mitokondria, the most important intracellular organelles in cells are necessary for the production of energy. On the other hand, it is known that during ischemia-reperfusion mitochondria death and damage to mitochondria play a crucial role leading to the release of the necessary cofactors to initiate cell death as well as the production and release of free radicals are damaging agents such as free radical (Lisa FD, Bernardi, 2006).

Thomas and colleagues showed that ischemia-reperfusion reduces blood flow and gastric mucosal prostaglandin E2 and enhances the freedom of expression and tumor necrosis factor-alpha and interleukin-B-1 plasma occurs. The effect of significant up regulation by mRNA, cyclo oxygenase 2 and capsaicin denervation is weak (Thomas, 2006).Andrew and his colleagues study the role of nitric oxide in the stomach against damage caused by ischemia-reperfusion.In this study, they reported that intravenous injection of sodium nitro prosayed or acetylcholine immediately before reperfusion reduces gastric mucosal injury (Andrews et al., 1994).

Salvia is a plant of the mint family; its scientific name is Salvia Officinalis. Seventeen thousand species of this plant species are unique to Iran, and Iran is the main habitat(Mozaffarian, 1996). Salvia rhytidea has multiple clinical health benefits and over the years has been used as a medicinal plant(Farhoudi et al., 2011; Akhondzadeh et al., 2003). Lamiaceae family includes a range of chemicals. Compounds such as terpenoids, phenolic and flavonoid and rankings are important. The family with which a phenolic acid also has anti-bacterial, anti-viral, anti-oxidant and anti-

inflammatory activities (Rafieian-Kopaei and Baradaran, 2013)

The antioxidant activity of flavonoids seems to be very important. In addition, flavonoids as an important factor to inhibit lipid peroxidation, platelet aggregation, control of capillary fragility and permeability and lipoxygenase enzyme activities have been identified. They can be applied for antioxidant strength and eliminate free radicals production (Middleton et al., 2000; Hosseinpour et al., 2013; Rafieian-Kopaei and Baradaran, 2013).

Flavonoids as part of the diet, absorption from the gastrointestinal tract can be followed through several mechanisms, antioxidant activity, removal of free radicals and lipid peroxidation inhibition is effective in preventing gastric mucosal lesions (Malek, 2004). Due to the antioxidant properties of various species of salvia rhytidea, research on histomorphometrical damage-ischemic reperfusion in rat's stomach and the possible effects of alcoholic extract of Salvia rhytidea the changes.

Methods Extraction

Salvia (leaves, twigs, flowers) and samples taken from Kerman were approved by botanists and then dried in the shade for ten days. 50 grams of dried herb in 250 ml of ethanol (96%) was added and kept for 72 hours at room temperature to complete soaking and extract compounds from the plant. Then it was flattened out on filter paper and distillation was done at low pressure until the solvent was removed and condensed extract was obtained. Finally, by completely drying the extract in an oven at 100 ° C the level of overall extract 7/56 percent W/W was determined.

Laboratory animals

Study on male Wistar rats weighing 300-350 g was performed. Animals in standard cages at 3 ± 22 ° C, humidity 5 ± 45% and photoperiodic period were maintained for 12 hours.18hours before the test animals were deprived of food but they were provided freely available water. All studies conducted on animals were in accordance with the instructions in the Care and Use of Laboratory Animals

The methods used for experiments

Classification and the implementation of the experiments were conducted as follows: rats were randomly divided into 7 groups, each consisting of 5 mice divided as follows:

Group 1) negative control: non-sampling ischemia.

Group 2) positive control: celiac artery ischemia in the stomach through close contact for 30 minutes and reperfusion for 30

or 60 minutes blood sampling after reperfusion.

Sham Group: manipulation of celiac artery without causing ischemia -reperfusion with normal saline and sampling.

Treatment groups:

1. The administration of aqueous extract of salvia rhytidea 3/2 mg / kg BW by gavage for 72 hours before ischemia and after 24 hours of sampling.
2. Administered aqueous extract of salvia rhytidea 3/2 mg / kg BW by gavage for 48 hours before ischemia and after 24 hours of sampling.
3. Administration of aqueous extract of salvia rhytidea3/2 mg / kg BW by gavage for 24 hours before ischemia and after 24 hours of sampling.
4. 4. Administration of aqueous extract of salvia rhytidea 3/2 mg / kg BW by gavage 0 hours before ischemia and after 24 hours of sampling.

Surgery for ischemia

After securing the animal proper anesthesia with ketamine 60mg / kg was administered to the back and the animals belly area was opened. Then to ischemia in animals celiac artery was taken using vascular clips. After 30 minutes, by releasing the clips blood flow is restored.

Animal rectal temperature was monitored during surgery by an electric heating pad kept at 37-38 ° C.

Sampling for the preparation of tissue sections

Following death without pain, abdominal and stomach area was separated in seven groupsand rinsed with saline, stomach was halved in the front of small and large curvature, one half of it was put in a container containing 10% buffered formalin for 24 hours, and after 24 hours cardia glands, gastric glands and pyloric glands were restored and transferred to 4% buffered formalin. 5 micron paraffin tissue slices were prepared and stained with H & E and green Masson trichrome stain. One gram of tissue was used with 5 ml phosphate buffer 0.1 molar for one gram of tissue. Good texture in Chinese mortar was crushed and mixed well with phosphate buffer then poured in a test tube, and the tube is centrifuged at 2000 rpm for 7 to 8 minutes. The supernatant is then placed in the freezer at -20 with Eppendorf. TAC Kit is then used for extraction and the plasma is measured by ELISA.

Histomorphometric study by optical microscopy

The study included the following parameters to standard methods micron thickness and

height and the number of cells per unit area is checkered by graticule.

Parameters studied included:

1-Thick layer of mucous, sub-mucous, muscular and serous stomach

2-The height of the gastric glands of the stomach pit

3-The size of the original cells, frontier and neck mucosa of the stomach gastric glands.

4-The number of primary cells, frontier and neck mucosa of the stomach gastric glands.

5-Measure the total antioxidant capacity of the stomach

6-Compare all the above in different areas of the gastric glands

Statistical Analysis

Data using statistical software SPSS-18 were analyzed and compared between treatments analysis of variance (ANOVA) and Tukey's test was used for diagnosis. Significance level of $p < 0.05$ was considered.

RESULTS

The results of this study are presented in Tables 1 and 2.

Table 1: mean, standard deviation, standard error and the minimum and maximum plasma antioxidant capacity in different groups of mice tested are shown.Results: Table 1 shows group 1 (negative control sample without ischemia), (positive control, causing ischemia and reperfusion in the stomach and sample 24 hours after reperfusion) and 3 (sham manipulation celiac artery without eschemia reperfusion with normal saline) with the group 7(Group therapy: Extract, 0 hours before ischemia and sampling after 24 hours) have a statistically significant difference ($p < 0.05$)

So the total antioxidant capacity of blood plasma in group 7, compared with the control group, sham or other treatments significantly increased.

Therefore salvia rhytidea extract which has antioxidant capacity compared with the sham and control groups was significantly increased ($p < 0.05$).

Although the increase in total antioxidant capacity in the other treatment groups was not significant this increase indicates that salvia rhytidea extract on plasma TAC is a function of time

Table 2 shows the mean, standard deviation, standard error and the minimum and maximum amount of the antioxidant capacity of the stomach extract tested in different groups of test mice.

Table 2 shows statistically significant difference ($p < 0.05$) between group 2 (positive control, create ischemia reperfusion), group 3 (sham manipulation celiac artery and normal saline) and 4

(treatment: Extract 72 hours before ischemia reperfusion).

Group 3 and group 5 (treating Extract 48 hours before ischemia reperfusion) and 6 (48 hours before reperfusion) shown significant difference ($p < 0.05$).

Group 5 with group 3 and 4 have a significant difference.

Group 6 with groups 3 and 4 show significant difference.

The results indicate that total antioxidant capacity changes in tissue extract a great deal of time has been receive extract.

The conclusion that can be derived from Table 2 is that group 3 with groups 5 and 6 showed statistically significant differences in antioxidant capacity and significantly increased in the groups 5 and 6

This means that salvia rhytidea extract in groups 5 and 6 significantly increased antioxidant capacity (the 3/48 to 16/83 in group 3, in group 5 and Group 6 has risen 19/33).

It noted the impact on the effectiveness of the salvia rhytidea extract on antioxidant capacity of tissues of group 3 to group 5 and 6, but with group 7 no significant difference was observed. Despite the fact that group 7 has increased the antioxidant capacity statistically significant differences were not seen and salvia rhytidea extract could work only at certain times in tissue extracts and cope with oxidative stress.

DISCUSSION

In the present study the effect of oral administration of extracts of Salvia officinalis L. Oral administration on histomorphometric changes induced gastric ischemia reperfusion in rats was studied. Based on the results of this study salvia rhytidea extract's antioxidant capacity compared with the sham and control groups significantly increased ($p < 0.05$).

Although the increase in total antioxidant capacity in the other treatment groups was not significant, it can be said that salvia rhytidea extract on plasma TAC is a function of time(Table 1).

Evidence from this study suggests that a large number of cells in the ischemic phase biochemical and pathological changes are still living cells and only a small population of cells at this time in the form of irreversible damage are seen. A group of cells damaged in the reperfusion phase will open its activities in the event that several of them go through necrosis (Maxwell & Lip, 1997). Construction of metabolic events that occur in cells during ischemia include: the loss of mitochondrial ATP stores because of injury during anoxia, with the loss of this energy source ATP-dependent ion pumps in the cell

will be disabled and, followed by cell swelling and irregular activities phospholipase and protease enzymes occur which can damage cell cytoskeleton (Brener, 2000).

By glycolysis in anaerobic conditions, lactate accumulation and acidosis occurs within the cell, but acidosis during ischemia, however, the cell has a protective role (Brener, 2000).

With the breakdown of calcium pumps in the cell membrane endoplasmic reticulum and calcium accumulate in the mitochondria, followed by activation of phospholipase enzyme occurring in mitochondria, leading to the release of toxic fatty acids. The cells are confronted with reduced glutathione and the antioxidant defenses of the cells are reduced. It should be noted that the full and effective reperfusion blood flow in the vessels will not be because of increased endothelial cells during ischemia and impaired release of nitric oxide (NO) (Noiri et al., 1996).As well as vascular endothelial cells is blocked by the accumulation of platelets and neutrophils space, normal physiology vessels will eat me)(Maxwell & Lip, 1997). Impaired endothelial cells lead to the release of oxygen free radicals during the reperfusion injury (Kadkhodaee et al., 1995).

Finally, due to the release of elastase and other proteases granulocytes activated from azurophilic granules will be accompanied by the creation of free radicals of oxygen. The production of free radicals leads to oxidative lipid membrane of cell.

Following the repression mechanisms of antioxidants such as glutathione in cells, during ischemia reperfusion greatly improved oxidative damage in cells. Following the repression mechanisms of antioxidants such as glutathione in cells during ischemia reperfusion oxidative damage to cells when the intensity of progress stems During reperfusion, oxygen free radicals and other oxidative damage cells, which are formed with a higher severity of ischemia, which mitigates the effects of rapid medical intervention to reduce ischemic necrosis and apoptosis depends.

One of the ways that can help reduce the negative effects caused by ischemia is the use of antioxidants(Lesson et al., 1988). Antioxidants in many experiments in laboratory environments and living environments-are employed and some of them have been investigated in clinical trials (Margaill et al., 2005).

Oxidation is a process in which the oxidant can be high in saturated fat, and in the body reacts by producing free radicals (peroxides), damaging the myelin sheath, lipids and

genetic material and other membrane components.Considerable evidence of the role of antioxidants in food are health maintenance such as a reduction of diseases caused by free radicals actions.

(Asadi et al., 2013; Asgary et al., 2013).

Several plants that offset the deleterious effects of hypoxia or ischemia have been identified. Salvia officinalis is the most valuable medicinal property of many drugs such as dark mint.Lamiaceae family has a wide range of chemical compounds including terpenoids, phenolics and flavonoids. Much research has been done on the effects of Salvia. Recent research has shown antioxidant properties, anti-inflammatory, anti-spasmodic, anti-anxiety, sedative, anti-toxic, Hypoglycemia, antimicrobial, analgesic and estrogenic have emphasizedsalvia rhytidea(Oboh& Henle, 2009; Hayouni et al., 2008; Arzi et al., 2011; Prodan&Tobra, 2010). This plant is used for the treatment of digestive disorders such as indigestion, constipation, stomach heaviness or weakness and nausea. Salvia rhytidea action stimulates the stomach, helps digestion and blood to flow faster (Zargary, 1990).

Flavonoids as part of the diet, the absorption from the gastrointestinal tract can be followed through several mechanisms antioxidant activity, inhibiting lipid peroxidation and scavenging of free radicals are effective in preventing gastric mucosal lesions(Malek et al., 2004).

In addition, flavonoids as an important factor to inhibit lipid peroxidation, platelet aggregation, control of fragility and permeability of capillaries, activities and lipoxygenase enzyme are known as cyclo oxygenase They can be applied for antioxidant strength and eliminate free radicals produced et al., 2000; Hosseinpour et al., 2013; Rafieian-Kopaei and Baradaran, 2013). Strong antioxidant activity of flavonoids against free radicals is their obvious ability to hydrogen (هیدروژن). (Rafieian-Kopaei, 2012).

It was suggested that plant antioxidants may prevent brain damage or brain damage caused by ischemia reperfusion treatment.So, it is interesting that the effects of antioxidants and were eliminated or trapping of free Radical for use as basic elements of protecting the brain against various damages to the brain, such as brain damage caused by ischemia - reperfusion has been studied(Baradaran et al., 2012).

CONCLUSIONS

The results of this study showed that the total antioxidant capacity changes in tissue extract

a great deal of time has been received and extract sage extract a certain time period could only have effect in tissue extracts and deal with oxidative stress.

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