Treatment Effects of Ginger Rhizome & Extract of Carrot seed on Diabetic Nephropathy in Rat

Khaki AA (Ph.D.)[1], Khaki A (D.V.M., Ph.D.)[2*], Ahmadi-Ashtiani HR (Ph.D. student)[3,5], Rastegar H (Ph.D.)[4], Rezazadeh Sh (Ph.D.)[5], Babazadeh D (D.V.M. Student)[1], Zahedi A (D.V.M., Ph.D.)[6], Ghanbari Z (M.D. Student)[7]

1- Department of Anatomical sciences, Islamic Azad University, Bonab Branch, Bonab, Iran
2- Department of Veterinary Pathology, Islamic Azad University, Tabriz Branch, Tabriz, Iran
3- Clinical Biochemistry, Tarbiat-e-Modarres University, School of Medical Science, Department of Clinical Biochemistry, Tehran, Iran & Biochemistry and Nutrition Department of Zanjan Medical University, Zanjan, Iran
4- Ministry of Health and Medical Education, Food & Drug Laboratory Research Center, Tehran, Iran
5- Institute of Medicinal Plants, ACECR, Tehran, Iran
6- Department of Veterinary Pathology, Islamic Azad University, Rasht Branch, Rasht, Iran
7- Medical school of Islamic Azad University, Tabriz Branch, Tabriz, Iran
*Corresponding author: Department of Veterinary Pathology, Islamic Azad University, Tabriz Branch, Iran, 1 Etekali La, South Shariati Street, Tabriz, Iran
Mobile Phone: +98-9143138-399
Email: arashkhaki@yahoo.com

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Abstract

Background: Antioxidants have essential effect on tissue regeneration after cells injury. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus. Ginger rhizome and carrot seed are strong antioxidants and long-term treatment of Streptozotocin induced–diabetic animals with these herbs, has been shown to reduce oxidative stress.

Objective: Evaluation to treatment effect of Ginger rhizome and extract of carrot seed on nephropathy after diabetes inducement.

Methods: Wistar male rat (n=70) were allocated into seven groups, control group, carrot seed extract group, ginger group, control- Diabetic group received 55mg/kg (IP) streptozotocin (STZ), treatment diabetic group that received carrot seed extract, treatment diabetic group that received ginger and treatment diabetic group that received carrot seed extract plus ginger. Animals were kept in standard condition. In 30 day after inducing diabetes, 5ml blood were collected for analyzing of TAC and MDA levels, and kidney tissues of Rats were removed in all groups then prepared for analysis.

Results: Pathological changes in diabetic group which received carrot seed and ginger together was decreased compared to control group. The rate of serum TAC significantly increased in diabetic groups which received carrot seed and ginger together significantly in comparison to control-diabetic group ($p<0.05$).

Conclusion: Since in our study 25 mg/kg carrot seed extract and 100 mg/kg ginger have prevented kidney tissue injury by reducing level of Reactive Oxygen Species (ROS) in serum, so it seems that using it can be effective for treatment nephropathy in Diabetic rats.

Keywords: Carrot seed, Ginger, Nephropathy, Diabetic Rats

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Introduction

Considering the current advances in the fields of histopathology, biochemistry and medical examination, the possibility of examining different factors on the body's organs, has been made possible treatment of enzyme-hormonal abnormalities concerning certain diseases such as metabolic diseases (diabetes). Of all the different possible factors, herbal ingredients could be a beneficial source both for research and showing successful compounds in this field [1, 2, and 3]. Reports show us that the number of diabetic and liver patients has been increasing over the years, which the different possible factors responsible for this increase could be genetic factors, systematic and infectious diseases, lifestyles (nutrition, use of different drugs, smoking and etc). Diabetes mellitus is a syndrome which by increasing the blood sugar causes abnormalities in the metabolism of fats, carbohydrates and proteins. It also raises the risk of possible vascular diseases [4]. The elevation in oxidative stress and changing level of anti-oxidants play a great role in the pathogenesis of diabetes mellitus [5, 6]. Although, the exact mechanism of diabetes mellitus is not yet well known, but the increased number of free radicals made by most of the mechanisms is harmful. diabetes mellitus is known as an important endocrine disease which disrupts the carbohydrate's metabolic balance. These changes increase the numbers of free radicals and oxidized LDL [7]. The presence of anti-oxidants such as vitamins and flavonoids can have beneficial protecting effects on the diabetic patients [8]. Currently the quercetin (a falavonoid) is known as a powerful anti-oxidant and in diabetic animals decreases the number of free radicals [9]. Studies on the chemical compounds of carrot seeds and ginger shows that these plants contain a high level of anti-oxidants such as selenium, vitamins A, B, C, E, flavonoids, and glutathione [10]. Thus it can be proved that these ingredients can decrease the destructive effects of Streptozotocin on the liver tissue by decreasing the active oxygen species. Based on our previous studies, the success of ginger and carrot seeds as an herbal product in decreasing hepatic damages of diabetic rats was proved [11]. In this research we want to study the effects of ginger and carrot seed on the nephropathy induced by streptozotocin.

Material and methods

Animals

For this study 70 Wister breed *rats* were used which purchased from the pastor's institute center in Tehran. The rats were almost 8 weeks old and weighed around 220 $\pm$ 10 g. During the time of the research, these animals were placed in 12 hours of light and 12 hours of darkness (9 am to 9 pm.). The room temperature was kept at 24.6°C ± 0.7°C and the humidity level was measured at %55-60.

The rats were divided into seven groups, six of which for studying and one for controlling purposes. In the control group (n=10) the rats received 3 ml of distilled water daily in a gavages manner. In the carrot seed extract group (n=10), the rats received 100 mg/kg body weight daily in a gavages manner. The ginger group (n=10) received 100 mg/kg body weight daily in a gavage manner. The control diabetic group (n=10) received 55 mg/kg body weight daily of Streptozotocin by intra peritoneal injection. The first treatment diabetic group (n=10), which received carrot seed extract 25 mg/kg body weight daily in a gavages manner. The second treatment diabetic group (n=10), received ginger 100

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mg/kg body weight daily in a gavages manner. The third treatment diabetic group (n=10) received both ginger (100 mg/kg body weight) and carrot seed extract (25 mg/kg body weight) daily in a gavages manner. All animals in this study were killed after 30 days, according to the protection of the animal's law [13].

Making the extract

The gathered carrot seeds were shade dried with fresh air without, 100 grams of which were grinded and washed with diethyl ether in order to remove unwanted fats, and then the extract was made in a percolation manner for 24 hours with 500 ml of ethanol in a dark room. This stage was repeated 4 times; the extracts were cumulated, and then dried in reduced pressure by a rotary evaporator. The extract yield was 3.2% w/w. For dispensing of ginger, 100 mg of ginger was dispersed in 3 ml of purified water [12].

Measurement of Serum Total Antioxidant Capacity (TAC)

TAC was measured in serum by means of a commercial kit (Randox Co-England). The assay is based on the incubation of 2, 2-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) with a peroxidase (methhemoglobin) and hydrogen peroxide to produce the radical action ABTS+, which has a relatively stable blue-green color, measured at 600 nm. The suppression of the color is compared with that of the Trolox, which is widely used as a traditional standard for TAS measurement assays, and the assay results are expressed as Trolox equivalent (mmol/L), [13].

Measurement of Serum MDA

MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA formed/mL. Plasma MDA concentrations were determined with spectrophotometer. A calibration curve was prepared by using 1,1',3,3'-tetramethoxypropane as the standard[14].

Surgical Procedure

In the 30th day, (at the end of the treatment period), the rats were killed with diethyl ether, and kidneys tissues in control & experimental groups were immediately removed.

Statistical analysis

Statistical analysis was done using the ANOVA and test for comparison of data in the control group with the experimental groups. The results were expressed as mean ± S.E.M (standard error of means). P-value less than 0.05 were considered significant and are written in the parentheses.

Results

Results of Measurement of Total anti-oxidants capacity findings in Serum

The total anti-oxidants capacity found in the control's group blood was 0.70 ± 0.03 mill mol per liter , in the study groups from numbers two to seven in order the amounts were equal to 0.75 ± 0.03, equal to 0.72 ± 0.04, equal to 0.41±0.05, equal to 0.60 ± 0.24, equal to 0.59 ± 0.54, equal to 0.69 ± 0.04 mile- mol per liter. Which our analytical and comparison survey shows significantly difference ($p<0.05$) in comparing the fourth study group and the control group.

Results of Malondialdehyde (MDA) levels in Serum

The Malondialdehyde (MDA) found in the control group's blood was 0.25 ± 0.03 mile-mol per liter, in the study groups from numbers two to seven the measures in order were equal to 0.22 ± 0.31, equal to 0.24 ± 0.03, equal to 1.1 ± 0.03, equal to 0.8 ± 0.03, equal to 0.8 ± 0.03, equal to

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0.5 ± 0.04 mile-mol per liter, which our analytical and comparison survey shows significantly difference (p< 0/05) in comparing the fourth group with the control group and the fourth group with the seventh group.

Kidney tissue findings in studying in light microscope

Kidney tissue studies in the groups receiving the carrot seed and ginger extract in comparison to the control group didn't show any pathologic changes, the proximal and distal tubules and glomerulus parts of the kidney were health from a morphologic point of view.

But in the diabetic-control group the hyperemia in glomerulus, bleeding in the cortical area of the kidney and degeneration of the proximal cells were also seen, these changes were less seen in the diabetic groups receiving the extracts, and in the diabetic group receiving the ginger extract and carrot seed extract the symptoms were less evident than the other groups.

Discussion

Diabetic nephropathy is the most common reason for causing ESRD, diabetic nephropathy starts with micro albuminoria, which is explained by the presence of (30 mg, 300 mg) of albumin in the urine per day. Unless people with type 1, diabetes and micro albuminoria are not treated, they will develop an 80% increase in nephropathy which is explained by secreting 300 mg of albumin in the urine. Although 20_40% of the people with type II diabetes will develop severe nephropathy in 15 years. There are different mechanisms both in the development and early formation of diabetic nephropathy, such as changing hemodynamic counteractions, metabolic changes and a genetic background [16]. Hemodynamic factors are same as the activities of different vasoactive systems, such as renin-angiotensin-aldosterone system (RAAS) and endothelin will increase in answering to the TGFβ1 secretion. Due to these reactions, hemodynamic changes such as systematic stress and inner stress of glomerulus are made. The metabolic pathway conflict (hyper-calcemia) causes the further increase non-enzymatic glycosylation, activity of the protein kinas C and the disruption of the POLYOL's metabolism. Numerous studies about the connection in the increased secretion of swelling molecules such as cytokines, growth factors, metalloproteinase have been proved. It also seems that stress-oxidative play a central role in diabetic nephropathy. Considering the current advances in the fields of histopathology, Biochemistry and Medical Examination, the possibility of studying effects of different factor on the body's organs for the treatment of enzyme or hormonal disorders concerning certain diseases such as metabolic diseases (diabetes) has been made possible. Of all the different possible factors, herbal ingredients could be a benefitting source both for researching and diagnosing useful compounds in this field [18]. Current reports show that the number of diabetic patients with kidney problems has increased which the different possible factors responsible for this increase in numbers could be genetic factors, systematic, infectious diseases, lifestyles (nutrition, use of different drugs, smoking and etc) [14, 15, 16]. Studies conducted on the chemical compounds of ginger and onion shows that they contain anti-oxidants [12, 17].Ginger contains vitamins, flavonoids which their antioxidant roles have been thoroughly been proved. The uses of ginger have been used as a cure for infertile men in traditional medicine that is mentioned in scientific sources. There are many compounds that their anti-diabetic

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effects have been proved such as quercetin, amines, peptides, lipids and flavonoids. Studies have shown that the liver controls the blood sugar level by glycogenesis and glycogenolysis which plays an important role in the metabolism of carbohydrates, and when the liver function is disrupted the metabolic homeostasis of glucose also becomes disrupted [19]. With the disruption of the glucose metabolism and the occurrence of hyperglycemia, connected genes by storing fatty acids in the liver's cells become active. The oxidative damages can be evaluated through measuring the Malondialdehyde (MDA) or oxygen free radicals, and also the conflict in the antioxidant defenses like the oxidation of LDL. Our studies showed that the measurement of antioxidant capacity in diabetic groups had decreased compared to the other groups which also resembled the other researcher's study [28]. The studies showed that the damages in the kidney tissue and death of the cells caused by diabetes were due to the increase in ratio of active oxygen species. Flavonoids can be found in fruits, vegetables, dark grapes and tea [20]. The level of flavonoids in the daily diet of a human is between sixteen to thousand milligrams per day. Our Perivisoly researches showed: Quercetin as a flavonoid that plays a crucial and important role in the human's diet. The beneficial effects of quercetin in human health are prevention from developing cataract caused by diabetes, decreasing vascular damages, anti inflammatory, anti microbial, anti virus, anti allergic , and preventing of blood clots formation. Another mechanism of quercetin is swallowing and cleansing free radicals such as xanthine oxidase and superoxide xanthine' [12, 14, 15]. Studies have showed the many quercetins doesn't have any harmful effects in the growth environment of the spermatogonial cells of the chicken, and causes an increase in the number of spermatogonial cells and decreases the oxidative effects. In this study carrot seed and ginger extracts decreases Malondialdehyde (MDA) and increases the antioxidant capacity levels in the serum which is similar to the previous studies [16, 17, and 18]. In order to decrease the complications of diabetic nephropathy in diabetic people, foods rich in flavonoids such as fruits, vegetables, dark grapes and tea is recommended.

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