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Antidiabetic and antioxidant effects of sage tea, *Salvia officinalis* in male rats exposed to hydrogen peroxide as a source of reactive oxygen species

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ABSTRACT

The antidiabetic and antioxidant effects of *Salvia officinalis* (sage tea) were studied in 20 mature male rats for 42 days. The rats were divided into four equal groups and given the following treatments: group one as a control group (C), rats only received tap water; group two (T_1) received 0.5% H_2O_2 as a source of reactive oxygen species (ROS); group three (T_2) received sage tea as 2 mg sage in 150 mL boiling water (ad libitum); and group four (T_3) received 400 IU/Kg BW/day vitamin E by oral intubation along with 0.5% H_2O_2. In order to determine serum glucose levels, blood samples were taken directly from the heart after the end of the experiment. Rats were slaughtered, and pancreas samples were collected for patho-histological analysis. The serum glucose levels in the sage tea with the H_2O_2 group (T_2) and T_3 as compared to the second group (T_1) showed a significant decrease ($p < 0.05$). Histological examination revealed significant recovery of the pancreatic tissues especially islets of Langerhans in sage tea and vitamin E rats (T_3). This investigation suggests the antidiabetic effect of sage tea for H_2O_2- induced diabetic rats mediated by their effect as antioxidants.

Keywords: *Salvia officinalis*, Hydrogen peroxide, Vitamin E, Type 2 diabetes mellitus, Rat.
Article type: Research Article.

INTRODUCTION

Glucose is the most important dietary carbohydrate and well known as the important metabolic full as well as the main source and storage of energy. So, its concentration in the blood should be maintained to meet the energy requirement of the body. The balance between the entrance of glucose from intestinal lumen into blood circulation and the uptake of glucose into the peripheral tissues which required sufficient level of insulin to stimulate the GLU T4 that mediate the uptake of glucose by the peripheral tissues leading to prevent the occurrence of hyperglycemia (Klover & Mooney 2004; Williams & Pickup 2004). The impairment in the synthesis of insulin by the beta cells is the main cause of diabetes mellitus which is regarded as the metabolic disorder occurred due to the failure of beta cells of pancreas to produce insulin as in type I diabetes (Insulin dependent) or due to failure in the sensitivity of body receptors to insulin as in type II diabetes (Non-Insulin dependent; Alberti & Zimmet 1998; American Diabetic Association 2010; Abhinov *et al.* 2013). The previous researches showed that oxidative stress occurs due to the disturbances between oxidant and antioxidant system leading to increased cellular activity for the production of reactive oxygen species (ROS) that exceeds the physiological capability of antioxidant defence system **(**Wilcox & Gutterman 2005; Khademian Amiri *et al.* 2022; Salim 2023). Increased blood glucose concentration plays an important role in the production of ROS and oxidative stress (Slatter *et al.* 2000; Deokar *et al.* 2016). So, the diabetes mellitus is one of the causes of increased oxidative stress, and it is regarded as one of factors that is a threat to the health (Adinortey *et al.* 2011). One of the most significant causes of oxidative stress and the generation of ROS in diabetes is hyperglycemia (Pierce *et al.* 2004). Under normal condition,

antioxidant as a scavenging molecule acts to prevent the over production of the reactive oxygen species (ROS) by converting them to H_2O. Synthetic or dietary supplements as a non-enzymatic antioxidant play an important role in scavenging the reactive oxygen species (Pierce *et al.* 2004). Due to their ability to transform ROS into water, scavenging molecules known as antioxidants like vitamin E are regarded as effective non-enzymatic antioxidants that help avoid the overproduction of ROS (Traber & Atkinson 2007). There is a huge of plants which are used as a good non-enzymatic antioxidant and antidiabetic through their effects on reducing the blood glucose (Li *et al.* 2013; Wang *et al.* 2013). Among these non-enzymatic antioxidants, *Salvia officinalis* which is rich in antioxidants, affects by providing a good protection against many disturbances due to its capacity to scavenger the free radicals (Miura *et al.* 2002) and exhibiting antidiabetic effect (Nunthaboot *et al.* 2013), which is considered by popular medicine as one of anti-diabetic plants (Alarcon Aguilar *et al.* 2002; Eidi *et al.* 2005), due to its ameliorative effect especially in those patient suffering from diabetes mellitus type 2 (Saxena & Vikram 2004; Lima *et al.* 2006;). The previous studies highlighted the effect of sage extract on hyperglycemia through using a different extract and experimental methodology (Alarcon Aguilar *et al.* 2002; Eidi *et al.* 2005; Eidi & Eidi 2009). In this study, mature rats exposed to oxidative stress caused by oral hydrogen peroxide (H_2O_2) injected for 42 days were tested for the antioxidant and hypoglycemic effects of sage tea (*Salvia officinalis*), the most common sage consumption in humans.

MATERIALS AND METHODS

Preparation of sage tea

Using 2 g of dried aerial plant material, 150 mL of boiling water was added, and the mixture was allowed to steep for 5 min. Sage tea was so created. This preparation produced by 0.1 mg mL^{-1} of extract infusion, with 1,8-cineole, cis-thujone, trans-thujone, camphor, and borneol as the major volatile components (4.8 g) and rosmarinic acid (362 mg mL^{-1}) of infusion and luleolin 7- glucoside (115.3 mg mL^{-1}) of infusion as the major phenolic compounds (Lima *et al.* 2006). By ingesting 0.5% H_2O_2 in drinking water for 42 days as a source of free radicals, mature male rats were given diabetes.

Experimental design

Twenty mature Wistar rats aged 2.5-3 months and with average weight of 220 g, randomly divided into equal four groups. Animals were kept in room maintained at a temperature of 23 °C for eight weeks (period of experiment), two weeks for adaptation to the experimental condition before treatment.

The rats divided into four groups as follow:

1. Rats of group one which received only tap water and served as control group (C).
2. Rats of group two which received 0.5% H_2O_2 in drinking water for 42 days (T_1).
3. Rats of group three which received sage tea Ad libitum plus 0.5% H_2O_2 for 42 days (T_2).
4. Rats of group which received orally 400 IU/ Kg BW of vitamin E plus 0.5% H_2O_2 in drinking water for 42 days (T_3).

Following the completion of the 42-day experiment, all of the rats were given ether anaesthesia before having blood samples taken directly from the heart and split into serum and glucose estimates (Weekers 2003). Then the rats decapitated and sample of pancreas were preserved in formalin buffer solution for histopathological sections.

Statistical analysis

On the basis of one-way analysis of variance, statistical analysis of the data was carried out (ANOVA). The least significant differences (LSD) test was used to identify specific group differences with the significant level of 0.05 (Steel & Torries 1980).

RESULTS AND DISCUSSION

Our results revealed that the oral administration of 0.5% of H_2O_2 as a reactive oxygen species (ROS) in drinking water for 42 days causes significant increase ($p < 0.05$) in serum glucose concentration in treated rats (T_1; 118.32 ± 12.13) compared to control, third and fourth groups (C, T_2, T_3; 60.98 ± 4.14, 79.38 ± 13. 49 and 64.66 ± 2.86 respectively; Table1). Significant decrease ($p < 0.05$) was recorded in glucose concentration in both second and third groups (T_1 and T_2; 79 ± 13.49 and 64.66 ± 2.86) as compared to first group (C; 118.32 ± 12.13). The decrement in serum glucose concentration in third group (T_2) showed non- significant ($p > 0.05$) when compared

to control group (C). Non-significant decrease ($p > 0.05$) was recorded in serum glucose concentration between second and third groups (T_1 and T_2; 79.38 ± 13.49 and 64.66 ± 2.86 respectively).

Table 1. Serum glucose concentration (mg dL^{-1})

Parameter	Groups			
	C	**T_1**	**T_2**	**T_3**
Glucose Conc.	60.98 ± 4.14	118.32 ± 12.13	79.38 ± 13.49	64.66 2.86
	C	a	b	Bc

Note: Values are presented as mean ± SE (n = 5 / group); Letter denote significant differences between groups ($p < 0.05$); LSD = 20.72; C = Control group (only tap water); T_1 = 0.5% H_2O_2 with drinking water; T_2 = 0.5% H_2O_2 plus sage tea; T_3 = 0.5% H_2O_2 plus vit E

Several researchers have previously used a variety of experimental techniques to study the impact of sage extract on hyperglycemia (Alarcon Aguilar *et al.* 2002; Eidi *et al.* 2005; Eidi & Eidi 2009). Alarcon Aguilar *et al.* (2002) found that starved mildly diabetic mice due to injection of alloxan experienced a significant drop in blood glucose after receiving an intra-peritoneal injection of a sage water-ethanolic extract for four hours, but not severely alloxan-diabetic mice. It is well known that sage-tea-drinking increased the rat hepatocyte glucose consumption, this may be due to the decreased gluconeogenesis during fasting, as well as the inhibition effect of glucagon as a stimulator for the production of hepatic glucose leading to decrease in plasma glucose level (Li *et al.* 2004; Saxena & Vikram 2004). Thus, it cannot be completely ruled out that consuming sage tea will have an impact similar to that of metformin. Lima *et al.* (2005) noticed this effect when consuming sage tea for 14 days in mice and rat hepatocytes, which resulted in a drop in fasting glucose levels. Previous researches pointed out the effect of sage tea as antioxidants due to the significant increase in SOD and CAT activities (Scalbert *et al.* 2005). Therefore, H_2O_2 was used in our experiments as a source of reactive oxygen species. Such activity functions as an efficient stimulator for defense mechanisms or scavengers for the ROS as well as providing adequate protection for the cells against the ROS that are initiated by H_2O_2. Histological finding showed the normal structure of islets of Langerhans of pancreas (control group; Fig. 1-1), while the addition of 0.5% H_2O_2 in drinking water for 42 days showed marked atrophy of these islets (Fig. 1-2) and minimal fatty degeneration (Fig. 1-3). The architecture of the islets in rats treated with sage tea and vitamin E plus 0.5% H_2O_2 in drinking water appeared more or less like control group (Figs. 1-4 and 1-5) respectively. Plant derivatives with hypoglycemic properties have been used in flock and traditional healing system around the world (Alarcon-Aguilar et al. 2002). In the present study, effects of the antidiabetic antioxidants of *S. officinalis* (sage tea) was investigated in H_2O_2-induced diabetic rats. The results indicated that sage tea was effective in lowering hyperglycemia in rats exposed to 0.5% H_2O_2 as ROS.
Histologically, *S. officinalis* showed protective effect on pancreatic tissue specially islets of Langerhans similar more or less to that of vit E. It was obtained firstly by preventing the death of β cells, and secondly, by permitting recovery of partially destroyed β cells (Eidi & Eidi 2009). The prolonged treatment with sage may increase the number of insulin- producing cells of pancreas by revitalization or hyperplasia of β cells of H_2O_2-destructive islets. This may be explained that the pancreas has the ability to replace the destructive or lost cells as a result of containing stable cells that have the capacity of regeneration and proliferate to replace the lost cells (Lima *et al.* 2006; Sá *et al.* 2009). The antioxidant properties of sage tea may offer a good protection and prevention of progressive destruction in the *β*-cell of pancreas. So, it will provide long term protection of the *β*-cell (Scalbert *et al.* 2005). The previous study showed that the phenolic compound of sage stimulates the endogenous antioxidant defence systems which act as scavenger for the reactive species.

CONCLUSION

The current study revealed that the administration of *Salvia officinalis* showed significant hypoglycemic effect in hydrogen peroxide-induced diabetic rats. Such effect may be due to their antioxidant activity which is observed through the protective effect on pancreatic tissue specially islets of Langerhans similar more or less to effect of vit E. From the results of the current study, we recommend that the administration of aqueous extract of *S. officinalis* as sage tea beside the usual therapy exhibit a positive effect on lowering the effect of diabetes mellitus.

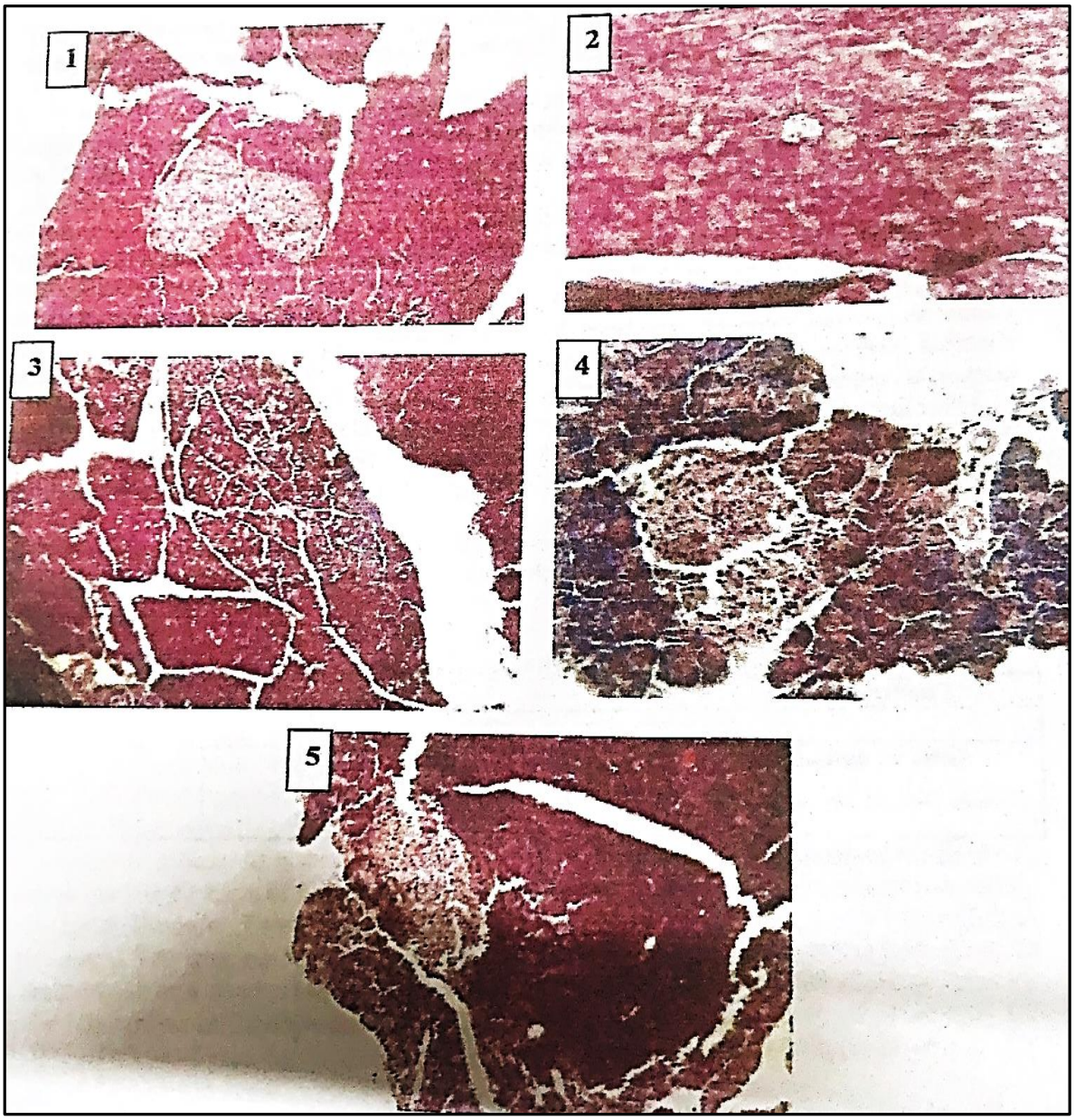

Fig. 1. Pathological sections of rat's pancreas stained by haematoxylin and eosin in untreated (control; 1) and 0.5% H_2O_2-induced diabetic (2), fatty degeneration of pancreatic rats treated with 0.5% H_2O_2 diabetic pancreatic rats plus sage tea (4) and diabetic pancreatic rats plus vitamin E (5).

REFERENCES

Alberti, KG & Zimmet, PZ 1998, New diagnostic criteria and classification of diabetes—again. *Diabetic Medicine*, 5: 535-536.

Abhinov, T, MdAasif, AK, Ashrafa, Parveen S & Kumar KP 2013, Diabetes epidemic in India: risk factors, symptoms and treatment. *Indian Journal of Research in Pharmacy and Biotechnology*, 1: 234-243.

Adinortey, MB, Owusu, RK, Galyuon, IKA, Ekloh, W, Owusu, I & Larbi, DA 2011, G-6-PD deficiency - A potential risk factor for development of diabetes mellitus. *Journal of Medicine and Medical Science*, 2: 1017-1021.

Alarcon Aguilar, FJ, Roman Ramos, R, Flores Saenz, JL & Aguirre Garcia, F 2002, Investigation on the hypoglycaemic effects of extracts of four Mexican medicinal plants in normal and alloxan-diabetic mice. *Phytotherapy Research*, 16: 383–386.

American Diabetic Association 2010, Diagnosis and classification of Diabetes mellitus. *Diabetes Care*, 33.

Deokar, P, Jagtap A & Yerawar, C 2016, Correlation of protein carbonyl and MDA in diabetes and its complications. *Indian Journal of Basic and Applied Medical Research*, 5: 284-289.

Eidi, M, Eidi, A & Zamanizadeh, H 2005, Effect of *Salvia officinalis* L. leaves on serum glucose and insulin in healthy and streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, 100: 310-313.

Eidi, A & Eidi, M 2009, Antidiabetic effects of Sage (*Salvia officinalis* L.) leaves in normal and streptozotocin-induced diabetic rats. *Diabetes & Metabolic Syndrome: Clinical research & Revies*, 3: 40-44.

Khademian Amiri, SA, Aghajanzadeh, T, Jafari, N & Mahmoudi, M 2022, Antioxidative compounds, enzymes activity and nutrient elements in Stachys byzantina are altered by climate conditions not by soil parameters. Caspian Journal of Environmental Sciences, 20: 1099-1115.

Klover, PJ & Mooney, RA 2004, Hepatocytes: critical for glucose homeostasis. *The International Journal of Biochemistry & Cell Biology*, 36: 753-758.

Li, R, Bilik, D, Brown, MB, Zhang, P, Ettner, SL & Ackermann RT et al. 2013, Medical costs associated with type 2 diabetes complications and comorbidities. *The American Journal of Managed Care*, 19: 421-430.

Li, WL, Zheng, HC, Bukuru, J & De Kimpe, N 2004, Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *Journal of Ethnopharmacology*, 92: 1-21.

Lima, CF, Azevedo, MF, Araujo, R, Fernandes Ferreira, M & Pereira Wilson, C 2006, Metformin-like effect of *Salvia officinalis* (common sage): is it useful in diabetes prevention. *British Journal of Nutrition*, 96: 326-333.

Miura, K, Kikuzaki, H & Nakatani, N 2002, Antioxidant activity of chemical components from sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) measured by the oil stability index method. *Journal of Agricultural and Food Chemistry*, 50: 1845- 1850

Nunthaboot, N, Lugsanangarm, K, Kokpol, S & Abd Elazem, IS 2013, Binding mode prediction of biologically active compounds from plant *Salvia miltiorrhiza* as integrase inhibitor. *Bioinformation*, 9: 426-431.

Pierce, JD, Cacker, AB & Arnett, MG 2004, Why should you care about free radicals. *RN*, 67: 38-42. https://pubmed.ncbi.nlm.nih.gov/14979192/

Sá, CM, Ramos, AA, Azevedo, MF, Lima, CF, Fernandes Ferreira M & Pereira Wilson, C 2009, Sage tea drinking improves lipid profile and antioxidant defences in humans. *International Journal of Molecular Sciences*, 10: 3937-3950.

Scalbert, A, Manach, C, Morand, C, Rémésy, C & Jiménez, L 2005, Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*, 45: 287-306.

Salim, SM 2023. Antioxidant activity of *Garcinia atroviridis* Griff. ex T. Anderson. *Caspian Journal of Environmental Sciences*, 21: 325-331.

Saxena, A & Vikram, NK 2004, Role of selected Indian plants in management of type 2 diabetes: a review. *The Journal of Alternative and Complementary Medicine*, 10: 369-378.

Slatter, DA, Bolton CH & Bailey, AJ 2000, The importance of lipid-derived malondialdehyde in diabetes mellitus. *Diabetologia, Springer-Verlag*, 43: 550-557.

Steel, RG & Torries, JH 1980, Principles and procedures of statistics. Biochemical approach, 2nd Ed., McGraw-Hill book Co., New York, USA.

Traber, MG & Atkinson, J 2007, Vitamin E, antioxidant and nothing more. *Free Radical Biology and Medicine*, 43: 1.

Wang, Z, Wang, J & Chan, P 2013, Treating type 2 diabetes mellitus with traditional Chinese and Indian medicinal herbs. *Evidence-Based Complementary and Alternative Medicine*, 343594.

Weekers, F 2003, Metabolic, endocrine and immune effects of stress hyperglycemia in rabbit model of prolonged critical illness. *Endocrinology*, 144: 5329- 5338.

Wilcox, CS & Gutterman, D 2005, Focus on oxidative stress in the cardiovascular and renal systems. *American Journal of Physiology-Heart and Circulatory*, 288: 3-6.

Williams, G & Pickup, JC 2004, Handbook of Diabetes, 3rd Ed. Malden: Blackwell Publishing.