

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

Sahib M.H. Mohammad Bakir^{1*}, Sadiq M. A. Al-Hiti², Rajiha A. S. Al-Naimi³, Dina T. Ali⁴.

1. Dentistry College/ Al-Bayan University, Baghdad, Iraq

2. College of Pharmacy, Baghdad, Iraq

3. College of Veterinary Medicine Baghdad University, Baghdad, Iraq

4. Institute of Embryonic Research and Infertility Treatment, Iraq

* Corresponding author's Email: sahib.mohammad@albayan.edu.iq

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 (T₁) received 0.5% H₂O₂ as a source of reactive oxygen species (ROS); group three (T₂) received sage tea as 2 mg sage in 150 mL boiling water (ad libitum); and group four (T₃) received 400 IU/Kg BW/day vitamin E by oral intubation along with 0.5% H₂O₂. 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 H₂O₂ group (T₂) and T₃ as compared to the second group (T₁) 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 (T₃). This investigation suggests the antidiabetic effect of sage tea for H₂O₂- 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 fuel 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 GLUT 4 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 H₂O. 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 scavenge 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 (H₂O₂) 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 luteolin 7- glucoside (115.3 mg mL⁻¹) of infusion as the major phenolic compounds (Lima *et al.* 2006). By ingesting 0.5% H₂O₂ 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% H₂O₂ in drinking water for 42 days (T₁).
3. Rats of group three which received sage tea Ad libitum plus 0.5% H₂O₂ for 42 days (T₂).
4. Rats of group which received orally 400 IU/ Kg BW of vitamin E plus 0.5% H₂O₂ in drinking water for 42 days (T₃).

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 H₂O₂ 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 (T₁; 118.32 ± 12.13) compared to control, third and fourth groups (C, T₂, T₃; 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 (T₁ and T₂; 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 (T₂) 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 (T₁ and T₂; 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); T₁ = 0.5% H₂O₂ with drinking water; T₂ = 0.5% H₂O₂ plus sage tea; T₃ = 0.5% H₂O₂ 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, H₂O₂ 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 H₂O₂. Histological finding showed the normal structure of islets of Langerhans of pancreas (control group; Fig. 1-1), while the addition of 0.5% H₂O₂ 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% H₂O₂ 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 folk 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 H₂O₂-induced diabetic rats. The results indicated that sage tea was effective in lowering hyperglycemia in rats exposed to 0.5% H₂O₂ 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 H₂O₂-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.

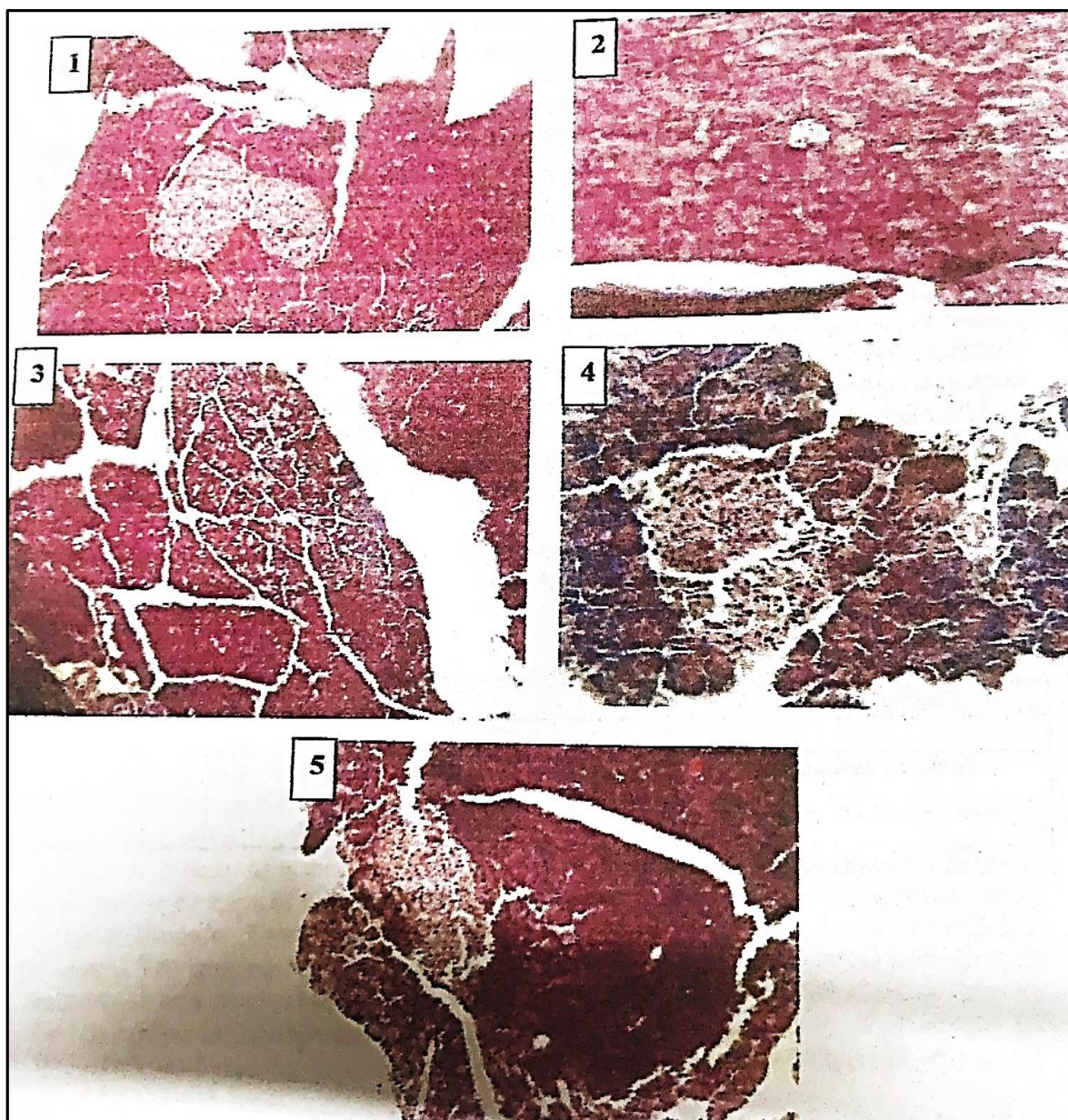


Fig. 1. Pathological sections of rat's pancreas stained by haematoxylin and eosin in untreated (control; 1) and 0.5% H₂O₂-induced diabetic (2), fatty degeneration of pancreatic rats treated with 0.5% H₂O₂ diabetic pancreatic rats plus sage tea (4) and diabetic pancreatic rats plus vitamin E (5).

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