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Influence of aqueous extracts from *Salvadora persica* L. (Chewing sticks) on lowering cholesterol in diabetics' male rats

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ABSTRACT

Salvadora persica or miswak sticks have been used as a medicinal purpose among global Muslim community. Various phytochemical studies on miswak or arak sticks reported the presence of antioxidant capacity, total phenolic acid, condensed tannins, total flavonoids compounds and reducing power in different extracts as ethyl acetate, methanol and diluted acetone. The results showed that the methanol extract had contained rich amounted phytochemical followed by diluted acetone and ethyl acetate.

Diabetes is the fifth leading cause of death in most developed countries. In spite of this, the drugs available in the market for treatment of diabetes are more expensive with side effect. Therefore, there is a high demand of cost effective novel natural antidiabetic drug without any side effect. In our investigation, we studied the effect of exact aqueous dosage 200, 400 and 600 mg Kg^{-1} body weight/ two day from *Salvadora persica* were oral injection separately in rats hypercholesterolemia. At the end of experimental period (four weeks), serum glucose and lipids profile were determined. The results illustrated that the diabetics and hypercholesterolemia rats in all groups were decreased gradually in body weight may be caused the aqueous extract from miswak had contained rich amounts in natural antioxidants. The lipids pattern were decreased may could be referred to multi factors beside the role of antioxidants may be playing a part of this action. Blood sugar was determined in diabetics and hypercholesterolemia rats fed on basal diet and oral injection separately from miswak aqueous extract at different dosage every two day and the results illustrated decreases gradually from 140.2, 130.1 to120.6 mg/dl, respectively.

From the obviously results it could be concluded and recommended that the aqueous extract from *Salvadora persica* had contained rich amounts in natural antioxidants and effect lowering diabetics and lipid parameters.

***Keywords*:** *Salvadora persica*, medicinal purpose, antioxidant, total phenolic, tannins, flavonoids, cholesterol, diabetics

Introduction

Miswak or arak (chewing sticks) is commonly used in the Arab and Islamic world for oral hygiene, teeth and medical, religious, and social purposes (Hattab, 1997). The miswak is important tool for oral hygiene and teeth in these countries. Recently, the World Health Organization (WHO) has recommended and encouraged the use of the miswak as an effective tool for oral hygiene and teeth. Arak tree is used as a diuretic, antigastric, to treat hook worn, venereal diseases, for teeth cleaning, amenorrhoea, in rheumatism, cough, and asthma, lowering cholesterol, reestablishment of the components of gastric mucosa, and as a laxative (Rotimi and Mosadomi, 1987).

Galati *et al*. (1999) reported that the *Salvadora persica* was significantly decreased in cholesterol and LDL levels in the rats, proving to be more active at 30 days of treatment. Also, the *Salvadora persica* was inactive at 18 h after treatment, whereas at 27 h it was able to reduce cholesterol and LDL plasma levels; in all the experiments were unchanged in HDL and triglycerides.

The beneficial effects of miswak in respect of oral hygiene and dental health are partially due to its mechanical action and partially due to pharmacologic action. Farooqi and Srivastava (1968) isolated benzyl-isothiocyanate from *Salvadora persica*, and they have found saponins along with tannins, silica, trimethylamine, and alkaloidal constituents.

Pharmacological studies showed that *Salvadora persica* plant possessed antimicrobial, antiplaque, aphrodisiac, alexiteric, antipyretic, astringent, diuretic, stomachic activities. It has great

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medicinal uses by herbalists and common man in in treatment of nose troubles, leucoderma, gonorrhea, boils, toothache, venereal diseases, lowering cholesterol and re-establishment of the components of gastric mucosa Galletti *et al.* (1993) and Alali *et al.* (2005).

Salvadora persica extract contains several organo-sulphur compounds and that certain sulphur derivatives is showed on hypoglycemic effect. Also, the herbal plant containing organo-sulphur compounds are used as a lowering of blood glucose Kupiecki *et al.* (1974).

Khan *et al*. (2009) showed that when the effect of different concentrations of miswak extract on L929 cell line in tissue culture and compared the results with sodium hypochlorite (NaOCl). The concentration was dependent morphologic change of L929 cell line when exposed to miswak extract and NaOCl. Moreover, the cells after a 4-h exposure period was suspected recovery to different miswak extract concentrations Al-Otaibi *et al*. (2004).

Amin and Al-Abad (2008) reported that the miswak extracte was significant decreased in phosphate and pH and it was significant increasing in calcium (22-fold) and chloride (6-fold).Whilst, saturation of saliva with calcium inhibits demineralization and enhance demineralization of tooth enamel, whereas elevation concentration of chloride inhibits calculus formation in kidney. Darout *et al.* (2000).

The aim of this investigation was carried out to evaluate the minerals content, antioxidant activity and biological experimental effects from *Salvadora persica* aqueous extracts on lowering hyperlipidemic in rats by measuring blood glucose levels and lipid profiles.

Materials and Methods

Materials:

Dried plant sample was used in this study. Dry stems of *Salvadora persica* imported from local market were purchased from a local market. The sample was grounded by household grinding machine.

Methods:

Minerals content

The minerals content of *Salvadora persica* dried powder was analyzed by digesting 0.5 g of dry miswak powder in a HNO_3/H_2O_2 solution, according to the method described by Pequerul *et al*. (1993) and measured the minerals by ICP-MS (7500cx, Agilent, JP).

Salvadora persica extracts

Fresh plant samples were used. Extraction was made with three different solvent: a mixture of acetone: water (80:20; v/v), ethyl acetate and methanol. The extracts of the *Salvadora persica* were prepared by adding 4 g of small particle fresh plant material powder with a commercially available food blender to 40 ml solvent and allowing the mixtures to stand overnight at room temperature, after which the supernatants were filtered and then purified by fine mish then sterilized by Millipore filter papers 0.45µm and dried/evaporated under a controlled temperature (40°C), and their biological were evaluated.

Evaluation of total antioxidant capacity (TAC):

The assay is based on the reduction by the extracts according to the method described by Prieto *et al.* (1999). The antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW). The calibration curve range was 0 to 500 µg/ml. All samples were analyzed in triplicate.

Total phenolic content:

Phenolic content was assayed using the Folin-Ciocalteu reagent, following Singleton's method slightly modified by Dewanto *et al*. (2002). The absorbance was measured at 760 nm, after incubation for 90 min at 23°C in dark. Total phenolic content of leaves was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE.g-1 DW) through the calibration curve with gallic

acid. The calibration curve range was 0 to 400 µg ml^{-1}. Triplicate measurements were taken for all samples.

Total condensed tannins:

Condensed tannins were measured using the modified vanillin assay described by Sun *et al.* (1998). The absorption was measured at 500 nm against solvent as a blank. The amount of total condensed tannins is expressed as mg (+)-catechin g^{-1} DW.

Estimation of total flavonoids contents (TFC)

The total flavonoids contents were determined by aluminum chloride method (Chang *et al.,* 2002). The reaction mixture (3.0 ml) that comprised of 1.0 ml of extract (1:10 dilution), 0.5 ml of aluminum chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) was incubated at room temperature for 30 min and absorbance was measured at 415 nm. The total flavonoids contents were expressed in terms of ascorbic acid equivalent (mg/g) (Mervat *et al*., 2009).

Determination of reducing power (RP):

The ability of the extracts to reduce Fe^{3+} was assayed by the method of Oyaizu (1986). The absorbance was measured at 700 nm. The mean of absorbance values were plotted against concentration and a linear regression analysis was carried out. Increased absorbance of the reaction mixture indicated increased reducing power. EC50 value ($mg.ml^{-1}$) is the effective concentration at which the absorbance was 0.5 for reducing power. Ascorbic acid was used as positive control.

Biological experimental:

Preparation of aqueous extract

The stems sticks were cut into small pieces and ground in grinding machine to fine powder, mixed with distilled water, and extracted for 24 h at 150 rpm at 25°C in a shaker. The mixture was then centrifuged at 3000 rpm for 20 min. The supernatants were subsequently filtered through Whatman No. 1 filter paper and the filtrate was concentrated in rotary evaporator (Buchi Rotavapor R-200) at 70°C and was lyophilized. The resulting powder was packed in a glass bottle and stored at 4°C until needed. It was dissolved in distilled water to prepare the exact aqueous dosage (200, 400 and 600 mg Kg^{-1} body weight) for orally injection (Badruddeen *et al*., 2012). The extract obtained (4.56, 9.13 and 18.26% yield) was prepared in distilled water each time prior to experimentation.

Nutritional experiments:

Male rats (30 rats) weight ranging 140-155 g were purchased from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.. Animals were housed in individual cages with screen bottoms and fed on basal diet for eight days. The basal diet consisted of corn starch 70%, casein 10% corn oil 10%, salt mixture 4%, vitamin mixture 1% and cellulose 5% according AOAC (2010). After feeding on basal diet for eight days, rats were divided into two groups. The first group (6 rats) was fed on the basal diet for another four weeks (30 days) and considered as negative control. The second main group (24 rats) was fasted overnight and injected with streptozootocin (was dissolved in 0.1M citric acid buffer and adjusted at pH 4.5) into the leg muscle (5mg /100g body weight) to induce diabetic rats according to Madar (1983). After 48 h of injection the second main group was divided into four subgroups (6 rats for each). The first one (6 rats) was continued to be fed on basal diet and considered as positive control. From the second, third and fourth subgroups (6 rats for each) were fed on basal diet and oral injection separately from exact aqueous dosage (200, 400 and 600 mg Kg^{-1} body weight/ two day). Each rat was weighted every two days and the gain body weight was calculated.

At the end of experimental period (four weeks), the blood samples were taken with drawn from the orbital plexus and centrifuged at 3000 rpm to obtain the sera. After that, the sera were kept on a deep freezer at -20°C until their analyses.

Serum glucose, total lipids, total cholesterol and triglycerides were determined according to knight *et al.* (1972), Allain *et al.* (1974), Fossati and Prencipe (1982) and Tietz (1986), respectively. High and low density lipoprotein- cholesterol in serum was determined according to Burstein (1970) and Fruchart (1982).

Statistical analysis:

The obtained data were exposed to analysis of variance. Duncan's multiple range tests at ($P \leq 0.05$) level was used to compare between means. The analysis was carried out using the PRO ANOVA procedure of Statistical Analysis System (SAS, 2004).

Results and Discussion

Mineral content in *Salvadora persica*:

The minerals content profile of miswak showed high levels of potassium (12.198 mg/g) followed by sodium and calcium were 7.262 and 4.126 mg/g, respectively. Metals were also found in high concentrations and trace metals was the lowest concentrations in the dried powder of miswak are illustrated in Table (1). Miswak chewing sticks have been used for oral hygiene since ancient times (Dutta and Shaikh, 2012), providing clean teeth (Farooqi and Srivastava 1968), strong enamel) Akhtar *et al*., 2011), and protecting from pathogens that enter the body through the mouth (Khalil, 2006). The unique complexity of the Miswak phytochemicals and minerals, along with its long fibers, gives it an advantage as a tool for oral and dental health care through providing all of the necessary means of mechanical and chemical cleaning and maintaining healthy teeth and gums.

The release of chemicals and minerals from miswak at the time of usage stimulates saliva production and buffers its pH, which confirms previous reports of similar findings (Almas, 1993). As a consequence, the antimicrobial activity of the released phytochemicals reduces the total number of bacteria (Al-Bayati and Suliman, 2008).

Table 1: Minerals content (mg/g) and trace minerals (µg/g) found in *S. persica*.

Minerals	mg/g	Trace minerals	µg/g
Na	7.262 ± 0.002	Mn	9.26 ± 0.03
Mg	1.569 ± 0.001	Fe	174.42 ± 0.01
Al	0.051 ± 0.001	Cu	6.13 ± 0.01
Ca	4.126 ± 0.001	Zn	5.64 ± 0.02
K	12.198 ± 0.002	Pb	<0.003

Each value represents the mean ±SD.

Phytochemical in different extracts from *Salvadora persica*

Total antioxidant capacity, total phenolic acids, total condensed tannin, total flavonoids compounds and reducing power were determined in ethyl acetate methanol and diluted acetone extracts from *Salvadora persica* or miswak and the results are reported in Table (2). From the results it could be noticed that the antioxidant capacity of methanol extract from *Salvadora persica* was 5.23 and 1.63 folds higher than that of ethyl acetate and aqueous acetone extracts from miswak (329, 52.8 and 125 mgGAE.g^{-1} dry weight). This increase of antioxidant activity of methanol extract may be able to be the *Salvadora persica* extracts had contained high amounts of phenolics content and flavonoid compounds. The total phenolic acids was increased in the methanol extract (34.83 mg GAE.g^{-1} dry weight), while only12.4 and 4.43 mg GAE. g^{-1} dry weight was found in the aqueous acetone and ethyl acetate extracts of miswak. Similarly, total condensed tannin content in methanol extract was 3.25 and 2.09 folds increased than ethyl acetate and aqueous acetone extract (16.16, 3.9 and 5.23 mg EC.g^{-1} dry weight). Moreover, total flavonoids compounds was higher in methanol extract (8.15 mg AAE.g^{-1} dry weight) than ethyl acetate and diluted acetone were 3.94 and 4.68 mg AAE.g^{-1} dry weight, respectively. Meanwhile, the results from reducing powder showed that the methanol extract from *Salvadora persica* or miswak was the highest than ethyl acetate and diluted acetone may be caused the methanol extract had contained rich amounts from natural antioxidant.

Our results agreement with the results obtained by Kornsteiner et al. (2006) that reported that the J. regia (bark) exhibited higher amounts of phenols in a ratio of 3.4 folds more. However, the results from walnut showed that the highest total phenolics content when different nuts extracted the phenolic fraction with a solution of 75% acetone and 25% of 526 µmol/L sodium metabisulfite. The differences in the results may be caused the different extraction methodologies. Moreover, Darout *et al*. (2000) and Abd-Rahman *et al*. (2003) reported that the *S. persica* higher amounts of flavonoids, salvadorine, cyanogenic glycosides, lignans, saponins, alkaloids, tannins, linoleic acid, steraric acid,

salvadourea, vitamin C, silica and different salts are also known to possess significant antimicrobial activity.

Table 2: Antioxidants activity in *Salvadora persica* extracts**.**

Antioxidant activities	Ethyl acetate	Methanol	Aqueous acetone
TAC: (mg GAE.g^{-1} DW)	52.8	329	125
Total phenolic acid: (mg GAE.g^{-1} DW)	4.43	34.83	12.4
Tannins : (mg EC.g^{-1} DW)	3.9	16.16	5.23
Total flavonoids (mg AAE.g^{-1} DW)	3.94	8.15	4.68
RP: EC50 (µg.ml^{-1})	176	940	299

Biological investigation:

Changes in body weight and daily feed intake:

The data concerning the changes in body weight and feed intake were determined in diabetics and hypercholesterolemia rats fed on basal diet and oral injection separately from miswak aqueous extract at different dosage every two day and the results are reported in Table (3). At the end of biological experimental, the results showed that the changes in body weight in case diabetics and hypercholesterolemia rats in control positive was decreased by -21.88 % followed by group 3 orally injection 600 mg Kg^{-1} body weight/ two day was -17.63%. Whereas, the group no., 1 and 2 showed lower in body weight (-8.71 and -9.55%) orally injection 200 and 400 mg Kg^{-1} body weight/ two day than healthy control negative (37.29) fed on basal diet. Moreover, the results from daily feed intake showed that the healthy rats in control positive was the highest in daily feed intake (12.54) followed by diabetics and hypercholesterolemia rats in control positive was 11.34. The diabetics and hypercholesterolemia rats in all groups were decreased gradually in body weight, may be caused the aqueous extract from miswak had contained rich amounts in natural antioxidants.

Table 3: Changes in body weight and daily feed intake on diabetics and hypercholesterolemia rats fed on miswak.

Groups	Initial body weight	Final body weight	Changes in body weight		Daily feed intake
			Gram	%	
Control negative	144.50±0.88	198.39±2.98	+53.89	+37.29	12.54±1.07
Control positive	154.84±2.92	120.53±1.49	-34.31	-21.88	11.34±0.43
Group 1	150.10±1.62	137.02±3.49	-13.08	-8.71	10.76±0.43
Group 2	152.92±2.46	138.31±1.94	-14.61	-9.55	9.39±0.65
Group 3	141.23±1.89	123.60±3.13	-17.63	-13.43	8.40±0.71

Each value represents the mean ±SD.

Effect of *Salvadora persica* on serum lipid patterns:

Total lipid, triglyceride, total cholesterol and cholesterol fractions were estimated in diabetics and hypercholesterolemia rats fed on basal diet and oral injection separately from miswak aqueous extract at different dosage every two day and the results are reported in Table (4). From the resultant it could be noticed that the total lipids, triglycerides, total cholesterol and LDL were decreased when the diabetics and hypercholesterolemia rats in the group (3) orally injection 600 mg Kg^{-1} body weight/ two day and the results were 198.70, 125.60, 94.37 and 44.26 mg/dl. The results in group 3 were nearly to the results from the healthy rats in control negative, 291.75, 119.72, 85.42 and 31.43 mg/dl. The decreasing in lipid pattern could be referred to multi factors beside the role of antioxidants may be playing a part of this action. Flavonoids are scavenging the free radicals and it can inhibit LDL oxidation in vitro and protection the LDL particles. Theoretically, flavonoids may be having protective action against atherosclerosis (Nijveldt *et al*., 2001).

Hyperlipidemia is a common disorder of lipid metabolism and it is the major cause for the atherosclerosis and coronary heart diseases (Choudhary *et al.,* 2005). *S. persica* has revealed that the diabetics and hypercholesterolemia effect marked by decline in the levels of TC, TG and LDL with concomitant elevation of HDL level in sera of rats treated with Miswak for 30 days. Moreover, Saini and Yadav (2013) and Khan *et al.* (2014) confirmed that *S. persica* exerts significant

antihyperlipidemic activity. A study by Kinosian *et al.* (1995) showed that, the changes in LDL/HDL and TC/HDL ratios were better predictors of coronary heart diseases than the changes in LDL alone. In the present investigation, miswak treated animals showed marked decline in the ratios of LDL/HDL and TC/HDL that demonstrates a possible protection against the risk of coronary heart diseases. *S. persica* contains flavonoids, which significantly increase LDL receptor mRNA levels causing increase in the rate of hepatic uptake and degradation of LDL leading to a decrease in serum LDL levels (Wilcox *et al.,* 2001).

Blood sugar was determined in diabetics and hypercholesterolemia rats fed on basal diet and oral injection separately from miswak aqueous extract at different dosage every two day and the results are reported in the same Table. The resultant showed that the blood sugar was 115.3 and 169.3 mg/dl in control negative and positive. Meanwhile, the different groups were orally injection separately from miswak aqueous extract at different dosage every two day the results illustrated decreases gradually from 140.2, 130.1 to120.6 mg/dl, respectively

Administration of *S. persica* aqueous extract for 30 days caused a significant decline in serum glucose level of the treated rats. The hypoglycemic effect of *S. persica* has been confirmed by previous investigators (Khan *et al.,* 2014). This effect may be attributed to the active compounds of *S. persica* that may facilitate peripheral utilization of glucose, either by direct stimulation of glucose uptake or by enhanced insulin secretion. In addition, the elevation in globulin concentration with Miswak supplementation and increasing globulin concentration over albumin concentration as indicated from the values of A/G ratio are indicators for increasing the immunity in Miswak treted rats. These findings are in accordance with Fortun- Lamothe and Drouet-Viard, (2002). Miswak includes flavenoids, certain alkaloids and polyphenolic compounds that seem to stimulate immune function (Ibrahim *et al.* 2005 and EI-Kholy *et al.* 2008).

Table 4: Means of serum total lipids, triglycerides, total cholesterol (mg/dl) in rats.

Groups	Total lipids	Triglycerides	Total cholesterol	LDL	HDL	Blood sugar
Control negative	291.75 ±5.77 ^{c}	119.72 ±6.00	85.42 ±8.61 ^{c}	31.43 ±4.11 ^{c}	79.86 ±1.91 ^{a}	115.3 ±5.7 ^{c}
Control positive	487.36 ±7.72^{a}	171.49 ±6.22 ^{c}	185.65 ±11.60 ^{a}	121.58 ±10.97 ^{a}	42.36 ±4.09^{c}	169.3 ±3.8^{a}
Group 1	324.91 ±4.98^{b}	149.04 ±4.16 ^{b}	142.92 ±6.69 ^{ab}	73.39 ±5.85 ^{ab}	64.33 ±3.73^{b}	140.2 ±3.5^{b}
Group 2	311.52 ±6.60 ^{b}	133.45 ±6.48 ^{b}	129.73 ±5.65 ^{b}	66.82 ±5.83 ^{b}	71.98 ±2.29^{a}	130.1 ±2.3^{b}
Group 3	298.70 ±6.83^{c}	125.60 ±9.58 ^{c}	94.37 ±6.78 ^{c}	44.26 ±5.60 ^{c}	75.18 ±3.36^{a}	120.6 ±1.2^{c}

Each value represents the mean ±SD. Mean followed by different superscript letters in each column are significantly different ($p<0.05$

Conclusion

Salvadora persica are rich source of secondary metabolites and minerals content which has powerful physiological effects in humans and are useful as medicines. Aqueous extract from *Salvadora persica* has great potential to lowering cholesterol in diabetics rats may be caused it had contained rich amounts from minerals content, natural powerful antioxidant and as an excellent total antioxidant capacity. Therefore, it seems the Salvadora persica plant is used for the therapeutic of diabetes and hyperlipidemia.

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