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Safety Profile of Meswak Root Extract on Liver, Kidney, Sexual Hormones and Hematological Parameters of Rats

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Abstract

This study was conducted to investigate the safety profile of *Salvadora persica* (*Salvadoraceae*) aqueous alcoholic root extract by carrying out acute and sub-chronic toxicity assessment in order to find out any side effect of the traditionally using of these root sticks. Regarding to acute toxicity test, mice were administered the extract up to 5 g kg^{-1}, intraperitoneally. Animals were then observed for behavioural changes; signs of toxicity, and mortality within 24 h. Surviving mice were monitored for 7 days for signs of delayed toxicity. In the sub-chronic toxicity test, rats were daily treated with the extract at a dose of 400 mg kg^{-1} intraperitoneally, for 30 days. At the end of the test period, hematological and biochemical parameters were determined in blood and serum samples with determination of vital organs weights. In the acute toxicity test, the extract was practically non-toxic showing no mortality and visible signs of delayed toxicity. The LD_{50}, given intraperitoneally, was estimated to be 4 g kg^{-1}. Administration of extract (at a dose of 400 mg Kg^{-1} b.wt.) to male and female rats for 30 days did not produce any significant ($P < 0.05$) effect on hematological and most biochemical parameters also vital organs weights. The root extract showed adverse effects on sexual hormones, by increasing estrogen secretion and reducing testosterone level in male rats. At the same time, the extract reduces progesterone level in female satellite group. Overall, Meswak aqueous extract is safe concerning liver and kidney functions and hematological assessments; however, it induces reversal effect on sexual hormones levels determined in sera.

Keywords: hormones, LD_{50}, root extract, *Salvadora*, safety profile

Introduction

Salvadora persica L, Meswak, is an ever green shrub and perennial halophyte capable of growing under extremely conditions. It is traditionally used in Islamic countries as tooth brush due to its antimicrobial and astringent properties.

The Meswak, a traditional chewing stick for cleaning teeth, is made from the plant *Salvadora persica*. Fruits have a sweet, agreeable, aromatic, slightly pungent and peppery taste. They can be eaten raw, cooked, or dried and stored. The leaves are also cooked as a sauce and eaten with couscous or as a green vegetable. For religious and cultural reasons, using Meswak is firmly established and widespread in most Muslim countries. It possesses high potential economic value as a source of oil and medicinal compounds (Maggio *et al.*, 2000). *Salvadora persica* contains many bioactive compounds such as benzylamides in stem extract (Khalil, 2003), flavonoid glycosides, luteolin-7-O-β-D-glucoside, apigenin rhamnoglucoside and rutin in ethyl acetate fraction of aqueous alcoholic extract (Ibrahim *et al.*, 2011a) as well as triterpenes, ursolic and oleanolic acids, in petroleum ether fraction of aqueous alcoholic extract (Ibrahim *et al.*, 2011b). Meswak has been reported to have many pharmacological effects such as antiplaque, anticaries, antiperiopathic, antiulcerogenic, anti-inflammatory, antimycotic, antidiabetic and antiviral properties (Almas, 1993, 1999; Darout *et al.*, 2000). Tooth brushing is universally accepted as a standard method to control plaque and calculus formation (Kakudate *et al.*, 2009). Several studies have reported on the antibacterial effects of chewing sticks, on carcinogenic bacteria, and periodontal pathogens, particularly bacteroides species (Wolinsky and Sote, 1983) and inhibitory action on dental plaque formation (Al-Lafi and Ababneh, 1995). Meswak is used in many developing countries as the traditional means for oral hygiene (Al-Otaibi *et al.*, 2004.) while it has adverse effects on male and female fertility (Darmani *et al.*, 2003). The different alcoholic and aqueous extracts have antiplasmodial activity against *falciparum* NF54 strain (Ali *et al.*, 2002) also extracts extended sleeping-time and decreased induction-time induced by sodium pentobarbital; in addition it showed protection against pentylenetetrazol-induced convulsion by increasing the latency period and diminishing the death rate (Monforte *et al.*, 2002). This study aims to find out the acute toxicity and safety of *S. persica* alcoholic extract prepared from root sticks of plants grown in Egypt.

Materials and methods

This study was conducted in National Research Centre, Egypt through 2009-2010.

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Plant

Salvadora persica was collected on April, 2009 from Aswan botanical garden and authenticated by the agriculture engineering of the botanical garden.

Preparation of aqueous extract

The fresh Meswak root sticks were cut into small pieces and allowed to dry at room temperature for fifteen days. Then it was ground to powder in a ball mill. The plant powder (2 kg) was exhaustively extracted with ethyl alcohol (70%), and then concentrated under reduced pressure using rotary evaporator till free from alcohol. The remained aqueous extract was lyophilized then was dissolved in a saline solution to prepare different extract doses (1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 g Kg^{-1} body weight) for intraperitoneally injection.

Experimental design

Acute toxicity test

The acute toxicity test for Meswak extract was carried out adopting the method of Bruce (1985).

Male albino mice were obtained from the Animal house of National Research Centre, Egypt. The animals were maintained under standard environmental conditions (23-25°C, 12 h/12 h light/dark cycle) and fed on standard diet and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee in National Research Centre. Animals were classified into two main groups, control group received 0.5 ml saline and treated animals comprising eight sub-groups administered 0.5 ml Meswak extract at doses of 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 g kg^{-1} b.wt. All treated groups comprising 6 animals each were fasted for 12 h prior to the experiment, and then they were given plant extract up to 5 g kg^{-1} in an intraperitoneal route. Mice were closely observed for 2 h post-treatment for behavioral changes and signs of toxicity. Mortality in each group within 24 h was recorded and surviving animals were observed for a further 7 days for any signs of delayed toxicity. The LD_{50} was estimated by log-probit analysis (Akindele and Adeyemi, 2006; Miller and Tainter, 1944).

Mid-term sub-chronic toxicity test

Male and female albino rats weighing between 150-180 g were obtained from animal house of National Research Center and they were divided into four groups comprising 8 animals housed separately per group. Female or male control groups were daily injected with saline (0.5 ml), however treated female and male groups were administered Meswak extract at one-tenth of LD_{50} for 30 days intraperitoneally (Orafidiya *et al*., 2004; Wurochekke *et al.*, 2008). Rats in different groups were weighed on 0, 7, 14, 21 and 30 days. They were closely observed for behavioural and general morphological changes. Animals were anaesthetized to facilitate collection of blood samples for haematological and biochemical analysis. Mortality in each treatment group was recorded in the course of the experiment.

Effect on vital organs

At the termination of treatment, 30th day, vital organs (heart, lungs, liver, kidneys, spleen and testis, male sex organs, were harvested from sacrificed rats. These were carefully examined for gross lesions and weighed (Precisa digital weighing balance [Type 300-9213/ E 125A], Switzerland) before preservation in 10% formo-saline. The weight of each organ was standardized to 100 g body weight of each animal.

Haematological assessment

Blood samples were collected from rats into EDTA (ethylenediamine-tetraacetate) bottles after superficially anaesthetized with ether. Collected samples were analyzed for determination of packed cell volume (PCV) (Dacie and Lewis, 1975), red blood cell (RBC) count, hemoglobin (Intern. Commit. for stand. Haemat., 1965), total and differential white blood cell (WBC) count percentage using standard methods (Ghai, 1995).

Biochemical assessment

At the end of the experimental period, animals were fasted for 12 h. All animals were superficially anaesthetized with ether and blood samples were collected from the retro orbital plexus, centrifuged at 3000 rpm for 10 minutes to separate sera. The liver enzymatic activity was determined according to the colorimetric method described by Hannig *et al.* (2009). Reduced glutathione concentration was measured spectrophotometry at 405 nm by the method of Plancarte and Hernandez (2004), while protein concentration (g dl^{-1}) was determined according to Okutucu *et al.* (2007). Total lipid concentration (mg dL^{-1}) was estimated by method of Vatassery *et al.* (1981). In addition, kidney function was assessed by measuring creatinine concentration (mg dl^{-1}) according to Demirovic *et al*. (2009), uric acid (mg dl^{-1}) by the method of Carolina *et al.* (2005). Also urea concentration (mmol L^{-1}) was estimated according to Yoneyame *et al*. (2001). Determination of sexual hormones in sera samples of male and female rats was assayed by radio-immuno assay (Nwafor *et al.,* 2007).

Statistical analysis

The results obtained were presented as mean ± SD while analysis of variance was performed by one way ANOVA procedure (SPSS 09.05) (Pipkin and Livingstone, 1984).

Results

Acute toxicity test

The aqueous alcoholic extract of *S. persica* did not produce any mortality when administered intraperitoneally up to 3 g kg^{-1} through 48 hr; and no visible signs of delayed

toxicity and mortality were observed when animals were monitored for a further 7 days. The LD_{50} was estimated to be 4 g kg^{-1}.

Sub-chronic toxicity study

Body weight

There were no significant differences in the weights of rats treated with extract at 400 mg kg^{-1} for 30 days compared to controls.

Effect on vital organs

Meswak extract did not produce any significant effect on the weight of various vital organs harvested from rats after daily administration for 30 days. Minor significant increase in female kidney weight was observed (Tab. 1). Generally, all increments or decrements in all internal organs were not significantly except liver, spleen and kidney weights in female satellite group.

Effect of Salvadora persica extract on haematological parameters at investigated dose

In female rats, *S. persica* at administered investigated dose did not produce any significant changes on haematological parameters (Tab. 2) when administered daily for 30 days. No significant ($P< 0.05$) changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume and differential white blood cell in the treated groups as compared to respective control group except significant increment was recorded in eosinophiles percentage which was increased by 38% over than the control.

In male rats, there are significant changes in WBC that increased by 21.5% and also observed with neutrophiles (16.5%) accompanied with elevation in eosinophiles percentage in blood, increment percentage was 53.5%. On the other hand, there was no significant increment or decrement in other haematological parameters.

Biochemical assessments

Serum glutamic-oxalacetic transaminase (AST) of animals treated with *S. persica* extract didn't exhibit any significant differences in female and male rats comparable to control group while administration of extract significantly decreased glutamic-pyruvate transaminase (ALT) by 15.10% and 14.02% for female and male rats, respectively.

Data presented in Tab. 3 revealed that *S. persica* extract increased serum protein production in female rats to be 6.21 g dl^{-1} while it decreased to be 6.93 g dl^{-1} in male rats as compared to control group, 5.77 and 7.23 g dl^{-1} for female and male rat, respectively. Glutathione level was significantly enhanced to be 3.22 mg dl^{-1} in female rat's serum and increased to be 3.9 mg dl^{-1} in male rats while controls were 2.48 and 3.74 mg dl^{-1} for female and male, respectively.

Kidney function assessed parameters (Tab. 4) showed the adverse effect of extract administration on release of uric acid in male and female sera as the extract reduces release of uric acid from 2.92 to 2.09 and from 1.68 to 1.39 mg dl^{-1} in male rat. On the other hand, treating experimental animals with Meswak extract for 30 days increased the release of urea in serum by about 9% in female and male rat while it decreased release of creatinine by 20.2% for female and by 1.3% in male rats.

Tab. 1. Effect of *Salvadora persica* aqueous alcoholic extract on rat organs weights (per 100 g body weight)

Organ	Heart	Lung	Liver	Kidney	Spleen	Ovary	testis
Male control	0.33±0.03	0.68±0.14	2.25±0.75	0.70±0.16	0.41±0.06	_	1.2±0.18
Treated male	0.32±0.04	0.69±0.11	2.22±0.73	0.73±0.15	0.39±0.07	_	1.3±0.11
Female control	1.07±0.03	1.30±0.02	5.72±0.16	0.98±0.04	0.67±0.02	0.10±0.00	_
Treated female	1.01±0.01	1.33±0.05	6.04±0.41 (5.6%↑)	1.11±0.03* (13.26%↑)	0.70±0.04	0.10±0.00	_

Values are expressed as mean ± SD, n = 8, *P<0.05

Tab. 2. Effect of *Salvadora persica* aqueous alcoholic root extract on haematological parameters in male and female rats

Parameter / Group	Hemoglobin (g dl^{-1}) (Increase or decrease %)	Packed cell volume (mm)	Red blood cell (x10^6/mm^3)	White blood cell (x10^3/mm^3)	Deferential white blood cell (%)				
					Basophile	Eosinophile	Neutrophile	Lymphocyte	Monocyte
Male control	13.32±2.1	39.87±3.14	7.45±1.3	9.65±0.99	1.6±0.03	1.7±0.01	22.09±1.4	72.01±2.85	2.6±0.04
Treated male	14.26±1.99 (7%↑)	37.77±3.51	7.02±1.1	11.73±0.86* (21.5%↑)	1.57±0.02	2.61±0.02* (53.5%↑)	25.74±1.52* (16.5%↑)	67.37±3.1	2.71±0.03
Female control	11.72±2.2	34.25±2.93	5.62±0.95	3.08±0.29	2.75±0.04	2.21±0.02	18.9±1.6	72.25±2.4	4.05±0.12
Treated female	12.85±1.88 (9.6%↑)	33.6±2.1	5.41±1.0	3.00±0.98	2.53±0.00	1.37±0.03* (38%↓)	17.11±1.43	74.98±2.3	4.01±0.21

Data are expressed as mean ±S.D., n = 8, *P < 0.05

Tab. 3. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), protein and glutathione concentration of rats administered aqueous alcoholic extract of *Salvadora persica*

Parameter / Groups	AST (U ml^{-1})	ALT (U ml^{-1})	Protein concentration (g dl^{-1})	Total lipid concentration (mg dl^{-1})	Glutathione concentration (mg dl^{-1})
Male control group	100.56±0.44	79.2± 0.83	7.23± 0.29	154.4± 0.11	3.74± 0.43
Treated male	102.06±0.66^{c}	68.1± 0.74^{a} (14.02↓)	6.93± 0.98^{b} (4.01↑)	155.7± 0.12^{c}	3.9± 0.26^{b}↑
Female control group	105.34±0.55	56.8± 0.8	5.77± 0.53	180.84±0.93	2.48± 0.31
Treated female	106.08±0.58	48.2± 0.3^{a} (15.10%↓)	6.21± 0.68^{b} (7.62%↑)	182.24±0.82^{c}	3.22± 0.55^{a}↑

Data are presented as the mean±S.D followed with increment or decrement %; a-P<0.01, b-P<0.05, c-n.s. compared to control group

Effect of Salvadora persica extract on sex hormones levels in serum of male and female rats treated with extract for 30 days

Male and female rats were treated with aqueous alcoholic extract of *Salvadora persica* (400 mg kg^{-1} b.wt) for 30 days then the blood samples were collected and sera were separated to estimate sexual hormones in response to extract using radio-immuno assay as mentioned before. The obtained data show that aqueous alcoholic extract of *Salvadora persica* at tenth of LD_{50} has different effects on male and female rats. It has no effect on estrogen levels in female serum while it significantly increased estrogen production in male rats to be 46 picogram (pg) ml^{-1} as mentioned in Tab. 5 against 36 pg ml^{-1} for control group. On the other hand, the extract at the same dose significantly (P<0.01) reduced the testosterone levels to be 0.5 nanogram (ng) ml^{-1} in male serum also significantly lowered progesterone levels to be 1.9 ng ml^{-1} in treated female rats against 4.22 ng ml^{-1} in control female rats.

Tab. 4. Serum levels of kidney function parameters of rats administered aqueous alcoholic extract of *Salvadora persica*

Parameter / Group	Creatinine concentration (mg dl^{-1})	Uric acid concentration (mg dl^{-1})	Urea concentration (mmol L^{-1})
Control of male	2.23± 0.83	1.68± 0.15	9.34± 0.56
Treated male	2.2± 0.82^{c} (1.3%↑)	1.74± 0.48^{c}	10.23± 0.19^{b} (9%↑)
Control of female	1.83± 0.11	2.92± 0.25	7.5± 0.11
Treated female	1.46± 0.39^{a} (20% ↑)	2.09± 0.7^{a} (28.43%↓)	8.02± 0.57^{b} (9%↑)

Data are presented as the mean±S.D. followed with increment or decrement %; a-P<0.01, b-P<0.05, c-n.s. compared to control group

Tab. 5. Effect of *Salvadora persica* aqueous extract on sex h*ormones* levels *in* serum of male and female rat treated with extract for 30 days

Hormone / Groups	Estrogen (pg ml^{-1})	Progesterone (ng ml^{-1})	Testosterone (ng ml^{-1})
Control male	36±2.3^{a}	-----	1.46±0.23^{a}
Treated male	46±0.8^{a}	-----	0.5±0.004^{a}
Control female	45±1.5^{c}	4.22±0.35^{a}	-----
Treated female	43±1.9^{c}	1.9±0.32^{a}	-----

Data are presented as the means±S.D, ; a-P<0.01, c-n.s. compared to control group

Discussion

The results obtained in acute toxicity investigation show safety of aqueous alcoholic root extract of *S. persica* when administered via intraperitoneal route. Administration of Meswak aqueous alcoholic extract up to 3 g kg^{-1} did not produce any mortality or visible signs of delayed toxicity. However, the LD_{50} was estimated to be 4 g kg^{-1}, therefore, it can be classified as relatively non-toxic extract. In this study, there were no reductions in body weight gain and gross examination of the organs in which minor insignificant changes in weight were observed did not show detectable abnormalities. In the case of the liver, there was no elevation in levels of associated enzymes to suggest toxicity.

In respect of vital organs in the mid-term sub-chronic toxicity study (30 days), some minor changes were observed but they are insignificant relative to the control group in all organs. Nonetheless, all of the increase and decrease were minor changes and the differences may have been due to the variation in size of internal organs and/or body weight of the animals as mentioned by Bailey *et al.* (2004) and Carol (1995).

The proportion of neutrophils was increased with an accompanying reduction in the proportion of lymphocytes with an elevation in eosnophile percentage in male rats in despite of no significant effects was observed on hematological parameters in female rats except with eosinophiles. All animals in male and female groups appeared normal and healthy, and no mortality was recorded all through the duration of the experiment.

Neutrophils are the first defense responders to microbial infections (bacterial or fungal) and are associated with other inflammatory processes. On the other hand, lymphocytes generate antibodies that bind to pathogens to enable their destruction and are more involved in defense against intracellular bacteria, virus infected cells and tumor cells (Adeyemi *et al*., 2010). The extract at this dose may be said to enhance immediate response to microbial attack and inflammatory processes.

Through the mentioned results it could be concluded that administration of *S. persica* extract for 30 days increased blood proteins production, synthesis of glutathione and release of blood urea while it decreased release of uric acid and creatinine in blood, in female rats. AST and lipid concentration showed insignificant changes in male and female satellites comparable to control group. Increases in the levels of liver enzymes marker, AST and ALT, above normal are reliable indices of liver toxicity (Hayes, 1989) or altered integrity of cellular membrane and cell death or lyses (Olagunju *et al*., 2000) that means the extract is safe for female rat at tested dose.

Concerning male treated animals, Meswak extract increased glutathione biosynthesis, ALT level and urea concentration while it decreases blood protein biosynthesis. On the other hand, AST and blood lipid production were remained as their levels in control. These results mean that *Salvadora persica,* chewing sticks, extract is totally natural product, cheap and safe, and it can be safely used for certain bioactivities. These findings are in accordance with Al-Koubaisi (2001).

AST is not specific for the liver only but is also important marker for other organs like heart, brain, kidney and skeletal muscle. ALT is the more liver specific enzyme for diagnostic use when the integrity of the hepatocellular membrane is compromised, there is extrusion of the enzyme into the plasma (Moss and Henderson, 1996).

The rise in the level of ALT is usually accompanied by elevation in the levels of AST which plays a role in the conversion of amino acids to keto acids, both AST and ALT are excellent markers of liver damage caused by exposure to toxic substances (Ranjna, 1999). Lowering the excretion of hepatocellular enzyme to serum as an effect of *S. persica* means its safety to liver cells.

It is generally acknowledged that the toxicity of liver cell is mediated by cytochrome P450 activity to generate a rather toxic metabolite, whose detoxification may lead to a dramatic depletion of hepatic glutathione (GSH). The results obtained revealed that administration of *Salvadora* extract has a hepatic protection properties in females that protect from GSH depletion with no significance differences between control or treated groups in the two animal gender, which go in parallel with those of Imberti *et al*. (2000).

Considering the effect of *Salvadora persica* aqueous extract on sexual hormones, the obtained results showed adverse effects on male and female rats. The extract significantly increased estrogen production and reduced the testosterone levels in male rat sera. However, the extract significantly lowered estrogen and progesterone levels in female rats.

The results presented are in coincided with Darmani *et al.* (2003) who stated that exposure of male mice to Meswak extract resulted in a 72% reduction in pregnancies in untreated females impregnated by testes males. Relative weights of the testis and preputial glands were significantly increased and that of the seminal vesicles was significantly decreased in test males. The obtained results showed that using the extract may cause prostate hyperplasia by increasing of estrogen and decreasing of testosterone. This phenomenon is the main reason for prostate hyperplasia as mentioned by Vahlensieck *et al.* (1996). The previous authors reported that benign prostate hyperplasia patients with stage II or III disease are suitable for drug treatment by alpha reductase inhibitors or aromatase inhibitors. The mode of action is reduction of testosterone level by conversion of testosterone to dihydrotestosterone and conversion of the testosterone to estrogen using anti-androgens. The results obtained go parallel with those of Darmani *et al.* (2003) who proved that *Salvadora persica* aqueous alcoholic extract may induce antiandrogenic activity.

Conclusions

The present results suggest that the hydroalcohol extract of *S. persica* is relatively safe when administered intraperitoneally in rats.

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