The Egyptian Journal of Hospital Medicine (Oct. 2015) Vol. 61, Page 445-450

Potential Effect of *Aloe barbadensis and Salvadora persica (Miswak) Mixture Sap* as a Contraceptive Therapy in Female Mice

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ABSTRACT

Background: Evaluation of herbs has been in progress worldwide for several decades to identify effective and safe substances for fertility regulation. This approach proved to be a good alternative to synthetic drugs as the chemicals of plant origin have limited side effects. Various medicinal plant extracts were investigated for their antifertility activity in female animal models.

Aim of the work: This study was designed to investigate the toxic effects of *Aloe barbadensis* and *Salvadora persica* (*Miswak*) *Mixture sap* and to assess them as a contraceptive therapy.

Material and Methods: Twenty female adult albino rats (Sprague dawley strain) were used in this study. Rats were divided into two groups (10 rats in each group); Group I (control untreated group) and Group II (mix treated group). *Mixture* of *Aloe barbadensis* and *Miswak sap* was orally administered (7 mg of *Miswak* + 7 mg of *Aloe* per 100 gram body weight) for 30 ± 2 days, where females were in the diestrus phase). All animals were decapitated after 30 days and blood samples were analyzed for estrogen, progesterone, tumor markers CA-15.3 and CA-125, kidney and liver functions, proteins profile and lipids profile.

Results: The mean serum level of estrogen was significantly increased ($p<0.01$), while that of progesterone was significantly decreased ($p\leq0.01$), in the *Aloe barbadensis and Miswak* group when compared to the control group. No significant difference was found between the treated and control groups for the serum level of tumor markers CA-15.3 and CA-125. Also, no significant difference was found between the two groups regarding kidney and liver function tests and proteins profile. The results also showed marked significant decline ($p<0.01$) in levels of the serum total lipids, total cholesterol, triglycerides, and LDL-cholesterol (LDL-C) in the treated group when compared to the control group. While, there was a significant elevation in HDL-cholesterol (HDL-C) level in the mix group when compared to the control group.

Conclusion: It could be concluded that *Aloe barbadensis and Miswak extract* can be used as a safe contraceptive therapy that can increase the estrogen level due to its phytoestrogen components such as beta sitosterol, without deleterious effects on the vital organs (liver and kidneys).

Keywords: *Aloe barbadensis*, contraceptive, fertility, kidney function, liver function, *Salvadora persica*, tumor markers.

INTRODUCTION:

Fertility control is an issue of national and international public health concern. There is a global need to support individuals in family-planning due to the increasing growth rate of the world's population with its negative impact on environment, economic growth, and poverty in underdeveloped countries.(1) Despite many achievements in human health care in the twenty first century, population in developing countries lack regular access to affordable essential drug.(1)

The cost of modern medicine is increasing by modern health technology and in many cases is inappropriate to the immediate needs of people in developing countries. Traditional medicine is sometimes the only affordable source of health care especially for the world's poorest patients. In addition, there is deficiency of systematic

Received:3/9/2015 DOI: 10.12816/0017697
Accepted:17/9/2015

plans for developing research capacity in traditional medicine leading to lack of a critical mass of traditional medicine researchers including traditional health practitioners.[2]

Several studies have shown that prolonged use of some of these pharmacological agents have a variety of moderate to severe side effects. Thus, the current research is shifting its focus to alternative therapies in order to prevent these deleterious effects. In India and China, many medicinal plants were used for the treatment of various diseases. The goal of herbal remedy is to enable the body to readjust excess levels of hormones, bring them to 'normal' and establish normal physiological function.[3,4,5]

Aloe barbadensis mill has been popularly used as a medicinal plant and is known for its hypoglycemic, lipid lowering, antiinflammatory, and antioxidant properties. Their previous work has already showed that an *Aloe barbadensis* mill gel (AVG) formulation caused partial reversion of estrous cycle and improved steroidogenic activity in the Polycystic Ovarian Syndrome (PCOS) rat model.[6,7,8]

MATERIALS AND METHODS:

This study was carried out in the animal house of Al-Azhar University (for girls).

Preparation of the Mixture:

- Five (5) grams of *Miswak (Salvadora persica)* and 5 grams of saber *(Aloe barbadensis mill)* were grinded and completed to 100 mL water and left overnight. Then the *Mixture* was filtered and kept in the refrigerator.
- The *Mixture* was prepared each two days.
- *Aloe barbadensis* and *Salvadora persica Mixture* extract was given daily between 7.00-8.00 a.m. during the experimental period.
- Twenty (20) female adult albino rats (Sprague dawley strain) were used in this study. They were acclimatized in the laboratory for one week. Their body weight was ranging between 130-140 grams.
- All animals were weighed 2 times; at the beginning of the experiment and before decapitation.
- Rats were divided into two groups (10 rats in each group). The first group was served as the control. While the second group was orally administered, through gastric tube, a *Mixture* of *Aloe* and *Miswak* (7 mg of *Miswak* + 7 mg of *Aloe* per 100 gram body weight).
- After 30 ± 2 days, while females in the diestrus, the 12 hours fasting animals were decapitated and blood samples were collected from both groups and sera were separated.

Hormones (estrogen and progesterone) were determined by using IMMULITE 1000 kits. Tumor markers for breast (CA-15.3) and for ovary (CA-125) were measured according to IMMULITE BR-MA (CA-15.3) kits and IMMULITE OM-MA (CA-125) kits, respectively. Other biochemical parameters in the serum i.e., kidney function tests, liver function tests, proteins profile, and lipids profile were measured by using biomerieux kits.

Statistical analysis:

Statistical analyses were carried out using the Statistical Package for Social Sciences for Windows version 22.0 (SPSS, Chicago, IL, USA). Descriptive statistics for each variable were determined. Results for continuous variables were demonstrated as data were expressed as Mean ± SE and compared differences among groups were identified by the Mann-Whitney *U* test; two-sided. *P-values* less than 0.05 were considered statistically significant.

RESULTS:

Estrous cycle of rats became irregular, with prolonged period of diestrus phase and reduced duration of proestrus, estrus, and metestrus phases after oral administration of the *Mixture*, with average estrus cycle length of two and half folds compared with the control group. Diestrus phase in the control group was approximately 2 days, but in rats treated with the *Mixture* aqueous extract it was elongated to 7 days.

Table (1): shows that animals treated with the *Mixture* had a significant increase ($p<0.01$) in estrogen hormone and a significant reduction ($p\leq0.01$) in progesterone hormone when compared to the control.

No significant difference was recorded in CA-15.3 (breast cancer marker) or CA-125 (ovarian cancer marker) between the *Mixture* treated group and the control group **(Table 2)**.

Table (3) shows that there is no significant difference in creatinine, urea, and uric acid between the *Mixture* treated and control group.

Concerning liver enzymes (AST, ALT and γ GT) activities, there was no significant difference between the control and plant extract treated groups **(Table 4)**.

Meanwhile, no significant differences have been recorded in the total proteins, albumin, and globulin levels, or in the A/G ratio between both groups **(Table 5)**.

On the other hand, *Mixture* treatment of the rats caused a significant increase ($p<0.01$) in HDL-C level when compared with the control rats. This was accompanied with a significant reduction of total lipids, total cholesterol, triglycerides, and LDL-C when compared with the control rats as shown in **(Table 6)**.

DISCUSSION:

Pharmaceutical plants are used in developing countries for many purposes. Plants are really important in drug production; many users of chemical drugs tend to use herbal drugs because they are safer than synthetic drugs.[9] Contraceptive drugs have many side effects specially that most of them cause breast cancer. So, women prefer to use medicinal plants instead. Folk medicine in Egypt offers many plants as contraceptive plants. Women prefer to use more than one plant to be sure that they will not get pregnant. The questions arise here are; would the usage of two plants have synergistic or antagonistic effect? Do they have interaction? Does this interaction lead to side effects? So, this study was planned to answer these questions.

Aloe vera and *Miswak* aqueous are the most favorite used plants for contraception. We prepared a *Mixture* of both plants in a simple manner as used in the folk medicine. The increase in the duration of diestrus may be due to temporary inhibition of ovulation.[10] *S. persica* (*Miswak*) has been shown to contain active chemical compounds as salvadorine, trimethylamine, fluoride, chloride, sulphur, silica, vitamin C, resins, saponins, tannins, flavonoids, and sterol.[3] *Aloe vera* contains anthraquinones, polysaccharides, glycoproteins, prostaglandins, and phytoestrogens such as beta cholesterol and campesterol.[4]

Phytochemical screening of medicinal plants revealed many bioactive agents as alkaloids, tannins, phlobatannins, anthraquinones, phenolics, flavonoides, saponins, and steroids. Alkaloids and flavonoids have reduced plasma concentrations of luteinizing hormone (LH), estradiol (estrogen), and follicle stimulating hormone (FSH). The enhanced levels trend to suppress the ovulatory cycle by inhibiting the secretion of both FSH and gonadotropin-releasing hormones (GnRH), which are necessary for ovulation.[5]

In the present study, there was significant increase in estrogen hormone accompanied with significant decrease in progesterone hormone when compared to the controls. This may be because *Aloe vera* extract contains compound that increase ovarian steroidogensis and serum estrogen concentration.[6] This extract has similar effects on FSH on the ovaries. This cause an increase in the growth and development of follicles and an increase in estrogen secretion from the follicular cells.[7]

Aloe barbadensis extract caused a reduction in serum LH concentration which could be explained by phytoestrogen influence on hypothalamus and an inhibition of activity of cells that produce GnRH, causing cessation of the hypothalamus hypophysis gonadal axis.[8] *Roberts et al.* showed that LH levels in rats exposed to genistein (a type of phytoestrogen) were reduced.[11] Since secretion of progesterone is dependent of LH, then with reduced LH, progesterone is also reduced.[12]

In the present study there was no significant difference in CA-15.3 (breast cancer marker) or CA-125 (ovarian cancer marker) between the treated group and control rats. This is because the *Mixture* extract contains phytoesterol, where high consumption of phytoesterol reduces the risk of leukemia, lung cancer, breast cancer, and fibrosarcoma. Reactive oxygen species (ROS) produced by oxidatively stressed cells can damage DNA, resulting in carcinogenesis, beta sitosterol increased the activities of antioxidant enzymes; superoxide dismutase and glulathione peroxidase,[10] where phytosterols can protect cells from damage by ROS. Phytosterols also can alleviate cancer development by reducing the production of carcinogens, where treatment with beta sitosterol decrease cell viability of leukemia, prostate and breast cancer cells, and fibrosarcoma.[13,14,15] Beta sitosterol caused an increase in Fas protein content and caspase activity which could be due to alteration of

structure and function of cancer cell membranes as a result of beta sitosterol incorporation into the membranes.

Phospholipids have been reported to interact more strongly with cholesterol than with phytosterols, indicating that the incorporation of phytosterols in the membranes can alter the structure of the membrane.(14) Cell membrane lipid rafts, in which sterols are highly concentrated, regulate cellular phosphorylation cascades that arise from external stimuli.(15) Therefore, incorporation of phytosterols into lipid rafts, altering their structure may result in beneficial differences in signal transduction. An additional mechanism through which phytosterols can act in promoting apoptosis is by lowering blood cholesterol levels. Reduction in blood cholesterol level could result in increased apoptosis.(16)

In the present study, there was no significant difference in kidney or liver function parameters in comparison with normal rats. In harmony with these results, proteins profile showed no significant difference. On the other hand, the present investigation showed hypolipidemic effect of the *Mixture* accompanied with an increase in HDL-C.

The liver plays an important role in lipid homeostasis. It is also involved in cholesterol synthesis and secretion of plasma lipoproteins. Hepatic LCAT (lecithin cholesterol acyl transferase) acts on lipoprotein remnants discharged in the plasma from the liver. It scavenges the cholesterol from these remnants so as to form HDL-C. Cholesterol can be then taken to the liver for degradation. *Aloe barbadensis* mill has been found to reduce level of LCAT activity and this result is confirmed by increase in plasma HDL-C levels.(17)

High LDL-C levels are usually associated with atherosclerosis. High HDL-C level reduce this risk. A possible mechanism of *Salvadora persica L.* extract may be due to the presence of flavonoids, which significantly increase HDL-C receptor mRNA levels, which in turn increase hepatic uptake and degradation of LDL-C causing a decrease in serum LDL-C levels. Also, it could be due to inhibition of intestinal absorption of cholesterol, degradation and interference with lipoproteins. Therefore, it could be hypothesized that *S. persien* enhances cholesterol excretion in the form of bile acid or of other sterols.(18)

From the ongoing discussion, our results have indicated that both *Aloe barbadensis* mill and *Salvadora persica L*. have synergistic effect for the hypolipidemic action.

We recommended using the water extract of both *Salvadora persica L.* and *Aloe barbadensis* mill as contraceptive *Mixture*. Where, it doesn't cause breast or ovarian cancer (as other contraceptive drugs do). Also, it doesn't affect vital organs as liver and kidneys.

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Table (1): Estradiol and Progesterone levels in the control and treated groups.

Groups / Parameters	Control	*Aloe barbadensis* + *Salvadora persica*	*P*-value
Estradiol (pg/mL)	17.26 ± 0.59	29.66 ± 1.02[a]	$p<0.01$
Progesterone (ng/mL)	15.82 ± 0.84	9.06 ± 0.44[b]	$p\leq0.01$

Values are represented as mean ± SE for each group of the ten animals.
[a]: significant increase compared to the control. [b]: significant decrease compared to the control.

Table (2): CA-15.3 and CA-125 levels in the control and treated groups.

Groups / Parameters	Control	*Aloe barbadensis* + *Salvadora persica*	*P*-value
CA-15.3 (kU/L)	0.02 ± 0.002	0.03 ± 0.002	$P>0.05$
CA-125 (kU/L)	0.02 ± 0.004	0.03 ± 0.004	$P>0.05$

Values are represented as mean ± SE for each group of the ten animals.

Table (3): Serum creatinine, urea, and uric acid levels in the control and treated groups.

Groups / Parameters	Control	*Aloe barbadensis* + *Salvadora persica*	*P*-value
Creatinine (mg/dL)	0.65 ± 0.06	0.68 ± 0.016	$p>0.05$
Urea (mg/dL)	32.08 ± 0.16	32.44 ± 0.18	$p>0.05$
Uric acid (mg/dL)	2.38 ± 0.09	2.18 ± 0.15	$p>0.05$

Values are represented as mean ± SE for each group of the ten animals.

Table (4): Serum liver enzymes activities (ALT, AST, and γ GT) in the control and treated groups.

Groups / Parameters	Control	*Aloe barbadensis* + *Salvadora persica*	*P*-value
ALT (IU/L)	59.6 ± 1.63	55.72 ± 3.21	$p>0.05$
AST (IU/L)	105.00 ± 8.25	103.00 ± 0.84	$p>0.05$
γ GT(IU/L)	2.28 ± 0.07	2.26 ± 0.21	$p>0.05$

Values are represented as mean ± SE for each group of the ten animals.

Table (5): Serum proteins profile parameters (g/dL) and A/G ratio in the control and treated groups.

Groups / Parameters	Control	*Aloe barbadensis* + *Salvadora persica*	*P*-value
Total proteins (g/dL)	6.72 ± 0.24	5.90 ± 0.21	$p>0.05$
Albumin (g/dL)	4.24 ± 0.04	3.66 ± 0.06	$p>0.05$
Globulin (g/dL)	3.16 ± 0.03	2.68 ± 0.17	$p>0.05$
A/G ratio	1.34 ± 0.02	1.41 ± 0.08	$p>0.05$

Values are represented as mean ± SE for each group of the ten animals.

Table (6): Serum total lipids (TL), triglycerides (TG), total cholesterol (TC), HDL cholesterol (HDL-C), LDL-cholesterol (LDL-C), and VLDL cholesterol (VLDL-C) parameters in the control and treated groups.

Groups / Parameters	Control	*Aloe barbadensis* + *Salvadora persica*	*P*-value
TL (mg/dL)	670.0 ± 0.13	574.0 ± 0.05[b]	$p<0.01$
TC (mg/dL)	139.80 ± 1.32	106.54 ± 5.81[b]	$p<0.01$
TG (mg/dL)	134.10 ± 0.51	124.82 ± 0.88[b]	$p<0.01$
LDL-C (mg/dL)	76.36 ± 0.46	66.94 ± 1.18[b]	$p<0.01$
HDL-C (mg/dL)	47.08 ± 0.34	56.52 ± 2.11[a]	$p<0.01$
VLDL-C (mg/dL)	26.62 ± 0.03	26.96 ± 0.18	$p>0.05$
TC/HDL-C	2.82 ± 0.03	2.41 ± 0.07	$p>0.05$
LDL-C/HDL-C	1.41 ± 0.02	1.37 ± 0.05	$p>0.05$

Values are represented as mean ± SE for each group of the ten animals.
[a] Significant increase compared to the control ($p<0.01$). [b] Significant decrease compared to the control ($p<0.01$).