Evaluate the effect of oral administration of Salvia officinalis extract on albino rats' fetuses during gestation period

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Abstract

Experimental studies on the toxicity of the sage are scarce despite its use in traditional medicine. So, this research is conducted to evaluate the toxicity of sage extract during the gestation period in female rats. 30 virgin female pregnant rats were divided into control and treated groups, 15 rats in each group. The treated group was received 500mg/kg rat of sage diluted in saline, from the 5th to 20th day of pregnancy. The control group received saline water at the same time. The gravid uteri of the pregnant dams were removed and its fetuses were extracted, then the fetal liver and kidney were stained with haematoxylin and eosin (H&E). The light microscopic examination of the fetal liver showed several hemorrhagic sites, rupture of endothelial cells lining the central vein with dilatation in the central vein and accumulation of the red blood cells and macrophages. Also, an increased in the number of erythroblasts and megakaryocytes are observed. Examination of the fetal kidney maternally treated with sage indicated that glomeruli appeared shrunken, swollen with wide, narrow capsular spaces respectively. The convoluted tubules show some abnormal changes in its histological organization; Nuclei of its epithelial cells are pyknotic with condensation in chromatin material. In addition, nuclei and cytoplasm of cells, which form the proximal and distal tubules show degeneration and necrosis on it. Salvia officinalis is should be considered as a drug and handled with precaution. Care must be taken to control the amount of plant extract taken by man.

Keywords : Kidney, liver, Rattus norvegicus, histology and Salvia officinalis

Introduction

The scientific word “herb” is derived from the Latin word herba meaning “grass”. This term has been referring to plants of which the leaves, stems, or fruits are used in several purposes such as food, for medicines, or for their fragrance or taste. Herbal medicine “herbology” refers to society and traditional medicinal practice depends on the utilization of plants and its extracts for the therapy of patient. There are many ways in which herbs can be administered, the most common of which is in the form of a liquid that is drunk by the patient - either an herbal tea or a (possibly diluted) plant extract (Bashar and Omar, 2011).

The herb under investigation is sage (Salvia officinalis) of family : Lamiaceae. Common sage (Salvia officinalis) is widely distributed in the northern coastal region of the Mediterranean and grows wild in the calcareous mountains of northern and central Spain, southern France, and the western part of the Balkan Peninsula. Common sage is cultivated in the countries of the Balkan Peninsula, throughout the Mediterranean region, and in the United States. In fact its name Salvia officinalis related to its medicinal characters. Salvia stems from the Latin word salvere, which means to save or to care. Also, the term officinalis is medical. This species of Salvia is familiar by woody stems and
blue and purple flowers. It is the most economically important species of the *S. officinalis* group and, along with *S. fruticosa* (Rivera et al., 1994), has a very long tradition as a medicinal and aromatic herb with a wide range of applications. Sage tea has been traditionally used as a therapy for digestive and circulation disturbances, bronchitis, cough, asthma, angina, mouth and throat inflammations, depression, excessive sweating, skin diseases, and many other diseases (Rami and Li, 2011; Walch et al., 2011; Khan et al., 2011). *Salvia* essential oils have been used as a therapy in a wide range of diseases like those of the nervous system, heart and blood circulation, respiratory system, digestive system, and metabolic and endocrine diseases. In addition, sage essential oil has been shown to have carminative, antispasmodic, antiseptic, and astringent properties (Loizzo et al., 2007 and Radulescu et al., 2004).

28 components were identified for *S. officinalis* (Hamdipour et al., 2014). By analyzed samples of *S. officinalis*, the major components, with different concentrations, are: 1,8-cineole, camphor, borneol, bornyl acetate, camphene, α- and β-thujone, linalool, α- and β-caryophyllene, α-humulene, α- and β-pinene, viridiflorol, pimaradiene, salvianolic acid, rosmarinic acid, carnosolic acid, ursolic acid, etc. (Croteau et al., 1981 and Khan et al., 2011). Studies have shown that some biological properties of the essential oil of *Salvia* depend on camphor, 1,8-cineole, α-thujone, and β-thujone (Radulescu et al., 2004). The essential oil of sage contains about 20% camphor, and as the leaves expand, the camphor content also increases (Avato et al., 2005). In a study, the most powerful scavenging compounds were reported to be α-thujone and β-thujone, bornyl acetate, camphor, menthone, and 1,8-cineol in the essential oil (Hussain et al., 2011). Several studies deal with phytochemical analysis of *Salvia officinalis*; changes in total phenolic, flavonoids and phenolcarboxylic acids (chlorogenic, cafféic, rosmarinic) contents of indigenous sage (*Salvia officinalis* L.) was investigated in different times of the year (Gîrd et al., 2014).

Research proves that aqueous extracts of *S. officinalis* has antiproliferative effects on two tumor cell lines. Also, it has cytotoxic and cytogenetic effects on tumor cell lines (Al-Barazanjy et al., 2013).

This current research is an attempt to review the usage of *S. officinalis* extract by pregnant albino rats and to discuss some of the implications arising from it in the kidney and liver tissues. The main aim of the study is to alert healthcare professionals to the fact that herbal medicine products are not entirely free of risks for pregnant women.

**Materials and Methods**

**Sample Collection and extraction**

A good quality, sage (*Salvia officinalis*) were purchased from spice dealer. Aqueous extract of *Salvia* leaves was prepared as follows: Amount of 50 g of the powdered plant was suspended in 200 ml of distilled water and stirred by magnetic stirrer overnight at 45°C, then was filtered once through gauze and once through filter paper. Filtrate extract was gently poured into pre-weighed glass Petri dishes and left at 37°C for 48-72 hours. The crude dried extract, then was placed in labeled, tightly sealed plastic tubes and stored at -20°C until used (Al-Barazanjy et al., 2013).

**Animal Housing**

Virgin female and male albino rats, *Rattus norvegicus*, weighing about 170-180g were used in the present investigation. The animals were obtained from the animal house of the Faculty of Veterinary Medicine, Giza, Egypt. Before starting the experiment, animals were allowed to acclimatize for at least 7 days. Pregnancy was established by housing females in the pro-estrous and estrous stages with healthy, fertile
males over night; 12-h light/dark cycle (2:1) under
controlled environmental condition of temperature (25
± 2°C), humidity (60 ± 20%) and given feed and water
ad libitum. The next morning, females with a positive
vaginal plug or vaginal smears were considered
pregnant, and the day of detection was defined as the
first day of pregnancy or gestation (McClain and
Becker, 1975). Experimental protocols and procedures
used in this study were approved by the Cairo
University, Faculty of Science, and Institutional Animal
Care and Use Committee (IACUC) (Egypt) (CUFS /
S /9/15). The animals were handled according to the
Guide for the Care and Use of Laboratory Animals, 8th

Experimental design and Animal treatment

Pregnant rats were allocated into two groups (15
per group). The control group received saline water
and the experimental groups received 500 mg/kg rat
of sage diluted in saline; according to Eidi et al. (2005),
the LD (50) of the methanolic extract of the Sage
is measured (4000 mg/kg). The herb was administered
orally by gavage from day 5 up to 20 of pregnancy,
deﬁned as the critical period for the structural
development span of the embryonic stage for rats
(WHO, 2001). The dams were euthanized with sodium
pentobarbital. Cesarean sections were performed on
the pregnant rats. The gravid uteri of the pregnant
rats were removed and its contents extracted (fetuses
with its corresponding liver and kidney).

Histopathological studies

Fetal biopsy of the liver, kidney and kidney of
the control and treated groups were firstly fixed for
histological investigation by light microscopy using;
10% formaline solution for 24 hrs. Washing was
done in running water to remove excess fixative
then dehydration using serial ascending series of
ethyl alcohol. Specimens were cleared in xylene and
embedded in parafﬁn wax at 56 degree in hot air oven.
Parafﬁn bees wax tissue blocks were prepared for
sectioning at 5 μm thicknesses using Reichert microtome.
The obtained tissue sections were mounted on glass
slides, deparafﬁnized, stained with haematoxylin
(Ehrlich) and counterstained by eosin for routine
examination, then examination was done through the
light electric microscope (Banchoft and Stevens, 1996).

Results

Fetal liver of untreated group

The liver of embryos of the control group displayed
the histological features of the normal liver (Fig.-1).

Histopathological Changes of liver tissue of fetus

The liver of this group showed a marked loss of
the lobular architecture and disorganization of the
hepatic strands. By examination of the liver of the fetus
maternally treated with sage revealed congestion of
the blood vessels and blood sinusoid, through which
blood escape, causing several hemorrhagic sites (Fig.-
2), also rupture of endothelial cells (EN) lining
the central vein is recorded with dilation in the central vein
(CV) with accumulation of the red blood cells and
macrophages (Fig.-3). Severe degeneration of hepatic
tissue is recorded; sign of degeneration was observed
by the presence of numerous vacuoles in the cytoplasm
of hepatocytes (H) and nuclear material of it (Fig.-4).
An increased in number of erythroblasts (EC) and
megakaryocytic (MG) are observed (Fig.-4).

Fetal kidney of untreated group

The embryonic kidney of the control group
displays the histological features of the normal renal
tissue (Fig.-5).

Histopathological Changes of renal tissue of fetus

Examination of the embryonic kidney of maternally
orally administrated sage reveals that, glomeruli appeared
shrunk, swollen with wide, narrow capsular spaces
Fig.-1: A Photomicrograph of a section of liver of a fetus from control mother. Showing normal liver tissue, the central vein with its intact endothelial lining (CV). The hepatic lobules that can be only distinguished by their central veins, hepatocytes (H) and blood sinusoids (Si).

Fig.-2: A Photomicrograph showing fetus treated liver, megakaryocyte (MG) and hemorrhagic sites (asterisks), central vein and endothelial cells (EN). Fig.- 3: A Photomicrograph showing fetus treated liver with degeneration in the endothelia lining the central vein, hepatocytes (H), erythrocytes (EC). Fig.- 4: A Photomicrograph showing fetus treated liver, hepatocytes, megakaryocyte and erythrocytes. Fig.- 5: Photomicrograph of a section of kidney of a fetus from control mother. Showing a part of the cortical region containing, a glomeruli (G) within Bowman's capsule (BC) and both proximal (x) and distal (D) tubules. Fig.-6: showing fetus treated kidney, decrease and increase in capsular space of Bowman’s capsule due to swollen and shrinkage in glomeruli respectively, necrosis (asters) in the proximal (PT) and distal tubule (DT), glomeruli (G), capsular space (CS), Bowman's capsule (BC). Fig.-7: Showing fetus treated kidney with pyknosis (P) to cells of distal and proximal tubules. Fig-8: Showing fetus treated kidney with degeneration of glomeruli of Bowman's capsule (arrow) and necrosis (asters). All photos have been stained with H&E stain.

Since the sage plant is widely used in traditional medicine, and regarded as safe by the U.S. Food...
and Drug Administration and is approved for food use as a spice or seasoning, any unreasonable utilization could lead to many complications caused by the well-known toxicity of the essential oil components of sage (Millet et al., 1981; Geller et al., 1984; Hooser, 1990; Leushner, 1997).

European Medicines Agency (2009) listed that the safety of sage during pregnancy has not been established, thus the use of it during pregnancy is not recommended. So, the current study was focused on the long term use of Salvia officinalis during pregnancy to evaluate its effect on the fetal tissues.

The present study has been observed that the impact of S. officinalis on rats during pregnancy causing many disorders on the fetal hepatic and renal tissues, but the plant sage were analyzed to twenty eight components (Hamdipour et al., 2014), to observe which of the sage components may be teratogenic.

Sage modulated antioxidant pathways to minimize stress by scavenging free radicals (Luvone et al., 2006; Elida et al., 2010), inhibiting lipid peroxidation (Ozkan et al., 2010). The active constitutes of sage polyphenols, especially, phenolic and rosmarinic acid have antioxidant effects, thus protecting membrane lipids of fatty acids and phospholipids from oxidative stress (Lima et al., 2005; Nour et al., 2010; Kianbakht et al., 2011). Also, Lima et al. (2007) who found that sage officinalis contain some phenolic compound and the most abundant ones are Rosmarinic acid and Luteolin-7-glucoside, which are characterized by a significant protection ability against cell death in the HepG2 normal cell line. The methanol crude extracts of six Salvia species were examined and the extracts were characterized by its antitumor character in vitro (Tepe, 2008 and Askun et al., 2009). There was a significant increase in proliferation of normal cell line; Murine fibroblast (L20B) as recorded by Al-Barazanjy et al. (2013).

The growth of tumor cell lines has shown a significant decrease. This significant decrease may be due to the effect of different phytochemical compounds found in S. officinalis, particularly triterpinoids as indicated by Al-Barazanjy et al. (2013), Slamenova et al. (2004) and Amirghofran et al. (2010) who found that different species of Salvia show inhibitory effects on tumor cell lines. The aqueous and methanolic extracts of Salvia triloba have a time dependent effect on RD tumor cell line as found by Ibrahim and Aqel (2010). The cytotoxicity of the plant extract could be due to presence of triterpinoids compounds in the extracts.

Salvinal isolated compound from aqueous extracts of Salvia miltiorrhizae, showed inhibitory activity against tumor cell growth (IC50 range, 4-17 μm) and induced apoptosis in human cancer cells. By using flow cytometry analysis, salvinal treatment resulted in a concentration-dependent accumulation of cells in the G2/M phase by inhibited tubulin polymerization in a concentration-dependent manners recorded by Chang et al. (2004).

Plant derived alkaloid flavopereirine, utilized in the form of a purified plant extract, selectively destroyed cancer cells. This selectivity of this anticancer agent could be due to the difference in membrane properties between cancer and normal cells reported by Beljanski (2000). This effect may be due to antioxidant activity and suppression of metabolic activation, which could be mechanisms through which sage or some of its components act as desmutagen. The antioxidant effect of S. officinalis was recorded by Patenkovic et al. (2009) and Dizaye (2010).

The ability of S. officinalis extracts to reduce the mitotic index (M.I) value can be traced to its chemical constituents such as taninnes that have the ability to block cell cycle progression Al-Barazanjy
et al. (2013). The study of Patenovic et al. (2009) and Grzegorczyk and Wysokinska (2008) explained the effect of different extracts of S. officinalis on M.I attributed to the antioxidant effects of terpenoids, which enhanced the cell death. In bone marrow cells of mice, reduction in the M.I exposed a mitodepressive ability of the extract of Sage on division Modallal et al. (2008).

Thujone as a one constitutes of sage is a monoterpeno that occurs mainly as a mixture of alpha and beta diastereoisomers, it lowers cholesterol and triglyceride levels (Kee et al., 2009 and El-Kholy et al., 2010). Any unreasonable utilization could lead to many complications caused by the well-known toxicity of the essential oil components of sage (Hooser, 1990 and Leushner, 1997). Mainly, such toxic effect has been attributed to terpenic ketones; the thujone and camphor components of the oil (Millet et al., 1981). Also, inflammatory properties to the oil extract are mainly due to the cineole component (Santos and Rao, 1997). Other side effects to the oils are hypoglycemic and spasmytic effects (Perfumi et al., 1991), epileptic reactions (Arnold, 1988 and Kbayssi, 1993), loss of equilibrium, tachycardia, permanent brain damage (Arnold, 1988; Newall et al., 1996) and other problems related to the nervous system (Elisabetsky et al., 1995). In animal studies, thujone as a one compound of sage extract inhibits the gamma-aminobutyric acid A (GABAA) receptor in the brain causing excitation and convulsions in a dose-dependent manner, there is some unclear data of carcinogenicity in rats (Pelkonen et al., 2013). Also, at dose-related pharmacotoxic symptoms were noted in mice upon injection of each of the four oil extracts. Farhat et al. (2001) recorded state of impaired coordination of movement and epileptogenic symptoms prior mice death. Highly vascularized skin and peritoneum layers, dark blood clots on the heart, and intestinal inflammation were recorded after death. Ingestion of three tablets of sage leaves per day by a 22-year-old young man resulted in epileptic convulsions, muscle cramps and respiratory disorders (European Pharmacopoeia, 1971), and implicated in abortion during pregnancy (Newall et al., 1996). Furthermore, Valnet (1990) recommended that this plant should not be consumed while lactation period because of its harmful effects on infants.

Conclusion

Salvia officinalis should be considered as a drug and handled with precaution. Care must be taken to control the amount of plant extract taken by man.

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References


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