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Investigating Effects of *Salvia Officinalis (*Sage) on Development of Mice Embryos Kidney and Some Hormonal Effect of Treated Mothers

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Abstract

Background *Salvia officinalis (sage)* is commonly employed in both medicinal and culinary preparations, *Salvia* plants are antioxidant and can improve 'head and brain' function.

Aim of study: To examine effects of aqueous plant extract of sage on development of mouse embryos kidney and hormonal effect of treated mothers.

Material and Method: Thirty adult albino Swiss female mice were used in this experiments study, their weight was 23-27 gm, three groups with 10 mice per group were categorized as:

G(A) orally treated with sage aqueous extract (167.8) mg/kg, G(B) administration orally with sage aqueous extract (83.9) mg/kg, G(C) orally treated with distilled water (control group) for 6 weeks. For histological study, the pregnant female mice have been sacrificed at day 17 of gestation of each group and 30 mice embryos were fixed in Bouin's fixative, paraffin infiltration. Specimen stains with H&E. After 24 hours from last orally dose of treatment period, 3 animals from each groups were sacrificed under ether anesthesia, 3 ml blood sample was collected from each animals, serum reproductive hormonal assay of FSH, LH, E2 and P4 were performed depends on kit assay procedure of ELISA kit.

Result :Histological examination of embryonic kidney after maternally oral administrated of *sage* reveals, different histopathological changes of embryonic renal tissue, also results revealed an increase that is highly significant *(P< 0.01)* in the numbers of fetuses belongs to G(A) and G(B) mothers at day 17 in comparison with G(C). Statistical analysis revealed in contrast with G(C) a highly significant *(P< 0.01)* rise in weights of fetuses (males and females) belonged to mothers of G(A) and G(B), while, serum hormones level show highly significant *(p≤0.01)* rise in E2 and P4 level in G(B) when compared with G(A), while significant decrease *(P<0.05)* in FSH level in G(A) and G(B), and there was a significant rise *(P<0.05)* in LH hormone level in G(A) and G(B).

Keywords: *Salvia officinalis (sage)*, aqueous extract, mice embryo.

Introduction

In the past years, *Salvia officinalis (sage)* Medicinal plants were regarded as the only form of healthcare accessible to the mainstream of human population [(1)]. Likewise, numerous plants was empolyed as sex stimulants in classical medicine [(2)]. It contains steroid and flavonoid such as genistein, daidzein and coumestrol [(3)] that are recognized as phytoestrogens. The metabolites of phytoestrogens act an estrogenic effect on central nervous system which stimulates cell division and growth of the genital tract of female animals [(4)]. *Sage* is similarly employed internally in the treatment of night sweats, excessive lactation, profuse perspiration (as in tuberculosis), excessive salivation (as in Parkinson's disease), depression, anxiety, menopausal problems and female sterility [(5)] (Fig.1), *Sage* likes being a panacea; it was employed as sexual debility; to treat mental and nervous conditions for menstrual and menopausal problems **[(6)]**. There are numerous bioactive compounds existing in this plant, like terpenes and phenolic compounds [(7)]and [(8)], that are believed to be the key for

various therapeutic properties accredited to sage [9].

Sega have antioxidant activities may be therapeutically helpful and it was revealed that the antioxidant potential of plants due to their phenolic components [10]. Nevertheless, their impact on preimplantation embryos does not have reasonable amout of studies to examine such impact [11]. The estrogenic activity of *sage* was demonstrated experimentally, the herb has strong effects in cases of oligomenorrhea and amenorrhea [12]. Therefore, it can be used as premenstrual tension, cysts, pelvic congestion fibroids, irregular (or heavy menses), and endometriosis for hormone imbalances, and it is not permitted to use in pregnancy [13].

Figure (1): Sage (*S. officinalis*) [5]

Materials and Method

Preparation of Plant Extracts

Plant extracts were done according to [11]. Leaves were air-dried and powdered using coffee grinder. Using soxhlet apparatus and water bath, 50 gm. of the leaf powder were removed for 3 H in 250 ml of the solvent (distilled water) at 45°C. Via a rotary evaporator, the leaf extract solution was evaporated at 45°C and the resulting crude cutting was held at -20°C.

Sage Doses and Concentrations: Aqueous plant extract was assessed in a mammalian model (albino mouse). Two doses of sage extract were used (83.9 or 167.8 mg/kg). These doses were correspondent to 10 and 20%, respectively of the dose in mice.

Laboratory Animals: Albino Swiss female mice were used in the experiments. Their age and weight at the beginning of experiments was 6-8 weeks, and 23-27 gm, respectively. Mice were grouped, each of which was kept in a distinct plastic cage. The mice were preserved at a temperature of 23 – 25°C, and had free access to food (standard pellets) and water.

Animals Groups: Effects of two doses (83.9 and 167.8) mg/kg of sage extract (aqueous) with control were investigated. Therefore, animals were grouped into three groups according to treatment procedure:

GA: Orally with the aqueous extract of sage (83.9) mg/kg (10 animals) for 6 weeks.

GB: Orally with the aqueous extract of sage (167.8) mg/kg (10 animals) for 6 weeks.

GC: With distilled water (control = 10 animals) for 6 weeks.

Blood sample collection and hormonal assay:

After 24 hours from last orally dose of treatment period, 3 animal from each group G (A), G (B), G (C) sacrificed under ether anesthesia, blood was collected from the animals in each group by cardiac puncture used disposable syringe of 5ml, blood samples were collected in a centrifuge tubes for maximum coagulation and separation of serum, after centrifuged at 3000 rpm/10minutes, serum was isolated, frozen at (-20°C) and then processed for hormonal assays. The serum reproductive hormonal assay of E2 and p4 were performed depends on kit assay procedure of ELISA kit (Germany).

In the metestrus phase, females remained with mature healthful males for mating (1 male/ 2 female). Vaginal plug incidence regarded as the initial day of pregnancy [14] the following days were consecutively counted. The pregnant female (PF) was extracted into separate cages.

When PF in G(A), G(B) and G(C) reach day 17 of gestation, animals undergo abdominal incision, plenty of died and alive fetuses in each horn were reported. Each fetus was washed and weighted for all groups. The embryos were stable in Bouin fixative and paraffin and implanted sections were stained with eosin and hematoxylin, two pathologists unaware of the plant and the dose individually read and reviewed the specimens.

Results

Daily administration of either 83.9mg/kg b wt. as G(A) and 167.8 mg/kg B wt. as G(B) of *Salvia officinalis (sage)* to female mice for 6 week until day 17 of gestation cause some changes including:

A-Weights changes: significant rise *(P< 0.01)* in fetuses weights (males and females at day 17 of gestation) in G(A) and G(B) in comparison with G(C) as showed in (Table 1). Differences recorded between body weight of males and females fetuses at right and left uterine horns in G(A) and G(B) administered mothers. These results showed that female's weights were higher than the males. These differences lacked significance (Table 1).

B - Numbers of fetuses: important rise *(P< 0.01)* in the numbers s of fetuses belongs to G(A) and G(B) mothers at day 17 in comparison with G(C) (Table.2).

C- Level of serum hormones: The result of hormonal level show a highly significant *(p≤0.01)* increase in E2 level in G(B) in contrast with G(A)as show in (Fig.2), Similar results seen in P4 level in G(B) in contrast with G(A) (Fig.3).

D - Histological Observations including:

1- **Control group (fetal kidney)**

The embryonic kidney of the control group displays the histological features of the normal renal tissue (Fig.4).

2- **Treated group(Histopathological Changes of kidney fetus)**

Fetus kidney shows that, glomeruli appeared shrunken, swollen with wide, narrow capsular spaces (CS) respectively (Fig.5). The convoluted tubules showed some abnormal changes in its histological organization; Nuclei of its epithelial cells were pykcnotic (P) with condensation in chromatin material .Also, an evidence of degeneration in glomeruli (G) of Bowman's capsule (BC) was recorded (Fig.5).

Table (1): Changes in fetal body weight at day 17 of gestation belongs to G(A) and G(B) treated with *Salvia officinalis (sage)* as compared with GC (Mean± S.E.).

Fetus at Day 17	Doses of *Salvia officinalis (sage)* (Mg/kg b. w.)	Weight of fetuses(g) Mean± S.E.	P value			
			Among groups	G(A) vs. control	G(B) Vs. control	G(A) vs. G(B)
Weigh of fetuses	control	0.471±0.038	≤0.001**	≤0.001**	≤0.001**	0.029*
	G(A)	0.41±0.03				
	G(B)	0.34±0.009				

NS= no statistical significant difference.

* =Statistically significant difference (P<0.05) .

** =Highly statistically significant difference (P< 0.01).

Table (2): Changes in numbers of male and female fetuses in left and right horns of uterus belongs to pregnant mice of G(A) and G(B) at day 17 of gestation as compared with control G(C) (Mean± S.E.).

	G(A)				G(B)				Control G(C)			
Horne	**No. of males**		**No. of Females**		**No. of males**		**No. of Females**		**No. of males**		**No. of Females**	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Left	16	33.33%	19	33.33%	22	46.8%	25	52.08%	35	51.47%	32	49.23%
Right	32	66.66%	38	66.6%	25	53.19%	23	47.91%	33	48.53%	33	50.76%
Total	48	100.00%	57	100.00%	47	100.00%	48	100.00%	68	100.00%	65	100.00%
Total male & Female fetuses	105				95				133			
P value	0.833				0.771				0.007			

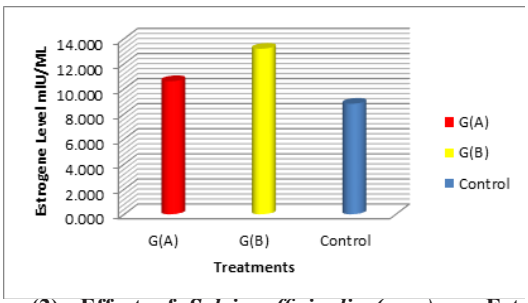

Figure (2): Effect of *Salvia officinalis (sage)* on Estrogen level(E4) in female mice serum.

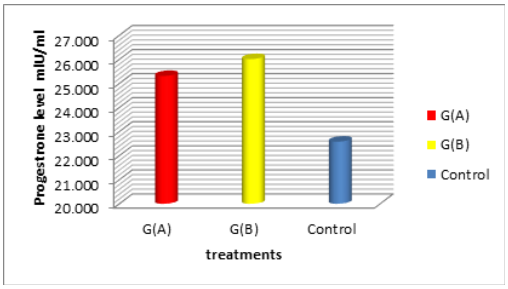

Figure (3): Effect of *Salvia officinalis (sage)* on Progestrone(P4) level in female mice serum.

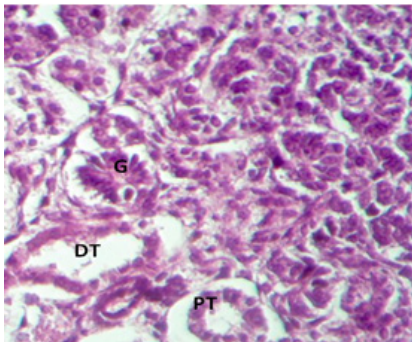

Figure (4): This slide show fetus Kidney of control group G(C) , cortical region containing, a glomeruli (G) within Bowman's capsule (BC) and both proximal (PT) and distal (DT) tubule.

Figure (5):This slides shows fetus treated kidney at day 17 of pregnancy ,histopathological changes of glomeruli tuft , decrease and increase in capsular space of Bowman's capsule due to swollen and shrinkage in glomeruli respectively (G) , nacrosis (asters) in the proximal (PT) and distal tubule (DT) capsular space (CS), Bowman's capsule (BC). (H&E, A400X, B1000X, C200X).

Discussion

The daily administration of aqueous extract of *salvia officinalis* as 83.9mg/kg b wt. as G(A) and 167.8 mg/kg B wt. as G(B) orally to pregnant female mice showed a significant reduction in fetal weight, these result agreed with that found by Evans *et al*[15] who proved that infants born to mothers with prenatal exposure to steroids had an increased risk of low birth weight. This reduction because *Salvia officinalis* contain steroid, isoflavones, and isoflavonoids [13] The mechanism of decreased fetal growth because progesterone possess both membrane and cytoplasmic receptors in a numerous reproductive and non-reproductive tissues including the brain and is a powerful inhibitor of GnRH for that exogenous progesterone may disturb the normal hormone work and interfere with normal genital development in fetal reproductive tract as demonstrated by Mari S [16].

In experimental groups G(A) and G(B), there were a highly significant decrease in fetuses number at day 17 of gestation and at birth time, this result explained on bases that progesterone make it harder for fertilized ova to be linked to the walls of the uterus, resulting in a lower probability of pregnancy as shown by Nagel[17].

Moreover, there are some differences in numbers of female fetuses which was higher in comparison with the numbers of male fetuses in G(A) and G(B), these results are due to that sex hormones powerfully impact the functional and anatomical organization of the sex organs, muscular and skeletal structure, and central nervous system (CNS), therefore is a major contributor to sexual discrepancy [(18)].

After 6 weeks of administration of *silva officinalis* , Preovulatory secretion of P4, even though restricted, can apply positive feedback on the estrogen-primed pituitary to enlarge LH release[(19)]. This phytoestrogen that is operating at high or low concentration disturbs diverse target tissues and performs as agonist or antagonist to E2. phytoestrogens have ability to affect rudimentary cell biology to their impact on cellular enzymes, in addition to their aptitude to impact growth factors, for instance, cytokines, and to control genes apply antioxidant actions even at a low level as shown by[(20)]. The Corpus Luteum secretes E2, P4, and inhibin, as documented by Knobil [(21)]. The estrogens use negative feedback at both low and high concentrations. On the other hand, progestins are operational just at high concentration [(22)].

Kidneys at second and third trimesters typically have the same configuration as in postnatal life, their appearance depend on the gestational age at normal configuration help in early diagnosis and optimize fetal safety then reduce the high prenatal morbidity and mortality[(23)]

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest : The authors declare that they have no conflict of interest.

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References

1. World Health Organization (WHO) General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines. 2000; Pp: 77.
2. Islam, M. W.; Tariq, M.; Ageel, A. M.; Al-Said, M. S. and Al-Yhya, A. M. Effect of Salvia haematodes on sexual behavior of male rats. *J. Ethnopharmacol*, 1991; 33:67-72.
3. Gruenwald J, Brendler T, Jaenicke C,Mehta MM, Fleming E, Hamid M and et al.(Eds.). *Medicago sativa* and *Salvia officinalis*. In: PDR for Herbal Medicines. 1st ed.,Medical Economics co., Montvale. 1998; pp: 963 - 1115.
4. S *LiebermanAre* the differences between estradiol and other estrogens, naturally . **J. of *Clinical Endocrinology* and *Metabolism***, *81* ;*1996* , pp. *850*-851
5. Baricevic D , Sosa S, Della Loggia R, Tubaro A, Simonovska B, Krasna A, Zupancic A. Topical anti-inflammatory activity of Salvia officinalis L. leaves: the relevance of ursolic acid. ***J Ethnopharmacol.*** 2001;75(2-3):125-32.
6. Dweck, A. C. The folklore and cosmetic use of various Salvia species. In: Kintzios, S. E. (Ed.), SAGE - The Genus Salvia. Harwood Academic Publishers, Amsterdam 2000; , pp 1-26
7. Cuvelier, M. E. Richard, H. and Berset, C. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage rosemary. ***J. Am. Oil Chem. Soc***. 1996; 73: 645-652.Amsterdam, Pp. 1-25.
8. Lima, C. F.; Andrade, P. B.; Seabra, R. M.; Fernandes-Ferreira, M.; Pereira-Wilson, C. The drinking of a Salvia officinalis infusion improves liver antioxidant status in mice and rats. ***J. Ethnopharmacol***., 2005; 97, 383-389.
9. Lu, Y. R. and Foo, L. Y.:Antioxidant activities of polyphenols from sage(Salvia officinalis).Food Chemistry, 2001; Vol.75 (2): 197-202.
10. Mates, M.; Ortiz-Lombardia, B.; Boitel, A.; Haouz, D.; Tello, S.; Usin, J.; Penninger, G.; Kroemer, P. & Al-Zari, M. Struct. Mol. Boil. 2002;9. 44-46.
11. Nadir, M. T., Salih, F. M., Dhahir, A. J., Nori, M. and Hussain, A. M..Antimicrobial activity of *Salvia* species indigenous to Iraqi. ***Journal BiologicalScience Research***, 1986;17: 109-117.
12. Salbei ,Master data ingredient / monograph Ingredient/plant: Sage (Salviaofficinalis) [monograph on the Internet]. Availablefrom:http://www.newpharma.ch/sites/default/files/newpharma/pdf/New_Pharma_Salvia_officinalis_monograph_eng0407.pdf. Accessedin: 2007; July 9th.2011.
13. Yardley, K. S. The role of herbal medicine in women's health [database on the Internet]. Medical Herbalist. . Available from:http://www.katolenyardley.com/Womens %20Health.pdf.

Accessed in: 2011; Aug 12th..

14. Roberts RV . The mouse: its reproduction and development. Animal Science1968*;* 54-60.
15. Evans AN, Brooke OG and West RJ. The investigation by pregnant women of substances toxics to the fetus. Practitioner 1980; 224: 315–319.
16. Mari S, Golub D, Farla L, Kaufman D ,Marlissa A, Campbell A and Ling-Hong L . Natural progesterone: information on fetal effects. Birth defects research part B: Rep. Toxicol.J.2006 ; 455–470.
17. Nagel SC . The effective free fraction of estradiol and xenoestrogens in human serum measured by whole cell uptake assays: physiology of delivery modifies estrogenic activity," Proceedings of the Society for Experimental Biology in Medicine 1998 ; 217: 300–309.
18. Morris JA , Jordan CL and Breedlove SM . Sexual differentiation of the vertebrate nervous system. Nat Neurosci 2004 ; 7:1034–1039.
19. Vander, A., Sherman J., Luciano, D. Female reproductive physiology. In:Human Physiology: The Mechanisms of Body Function, 7th ed., WCB / McGraw- Hill, New York, Missouri 1998;649 – 663.
20. Brown, N.M. Setchell, K.D.R. Lab. Invest. 81,2001; 735 – 747.
21. Knobil E., Hotchkis J. The menstrual cycle and its neuroendocrine control. In: The Physiology of Reproduction vol. 2, Raven Press, New York 1988; 1971 – 1994.
22. Matikainen T., Ding Y.Q., Vergara, M., Huhtanieni, I., Couzinet, B., Schaison, G. J. Clin.Endocrinol. Metabol. 75 ,1992;820 – 825.
23. Mohamed Adam, Jumaa Yousif Tamboul , Mohamed Yousef ,Abdelmoneim SuliemanThe normal fetal kidney measurement in normal pregnant ladies (Journal of Amaricane Science 2013:9(12).