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Effect of Ethanolic Extracts of *Salvadora persica* Roots on Female Albino Rats.

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ABSTRACT

This study was performed in the animal house of the faculty of veterinary medicine and animals and plants laboratories of the Department of biology in Faculty of Science / University of Kufa for the period from September 2014 to February2015 to investigate the biological activity of hot aqueous and ethanol alcohol extracts of *Salvadorapersica*plant roots (*Salvadorapersica*)as antifertility in female albino rats (*Rattusratus*L.) .In this study 85 rats were used aged form 8 to 12 weeks and with daily dosage orally by concentrations (250 , 500) mg/kg of body weight of both extracts for periods (10 , 20 and 30) day was given.The results of the current study indicated a significant decrease ($p<0.05$) in the weight of the ovaries and a significant increase ($p <0.05$) in the weight of the uteruses for both concentrations and dosing periods, two extracts compared with the control group.The current study revealed the occurrence of a significant decrease ($p <0.05$) in the level of each of follicle stimulating hormone (FSH) and luteinizing hormone (LH) and significant increase ($p <0.05$) in the level of estradiol hormone (E_2) for both concentrations and dosing periods and two extracts compared with the control group.The histological study showed occurrence of a significant decrease ($p <0.05$) in each of the diameters of the ovaries and the numbers of primary, secondary and Graafian ovarian follicles and the occurrence of significant increase ($p <0.05$) in the thickness of uterus for both concentrations and dosing periods and two extracts compared with the control group.

Keyword: *Salvador apersica* ,extracts, root , rats.

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INTRODUCTION

A good number of scientific papers have been already published related to the use of medicinal plants as antifertility . Some results investigated the anti-fertility actions of some medicinal plants in mice[1,2]and rats [3,4,5,6].*Salvadorapersica*belonging to the Family*: Salvadoraceaea* is a chewing stick called 'Miswak' and is one of the medical plants throughout India, also in the wider Muslim world [7]. *S.persica* (Miswak) is used traditionally in the treatment of rheumatism, leprosy, gonorrhea, ulcers, scurvy, tumors and dental diseases[8,9].It also participated to ritual purity*S.persica*also called (Arak) grow as a shrub naturally in the desert , also miswak bristles is used for brushing teeth also prepared head for brushing teeth [10]. Many species of these plant showed a contraceptive activity and estrogenic[11,12]. Some of animals that ingested plants possesses some of chemical active ingredients called phytoestrogens that are present in a wide variety of plants ,which may be ingested directly or as ingredients of tissues. Phytoestrogens have ability to reduce a fertility in domestic animals[4]. *S.presica*roots and bark consist of high ratio of alkaloids as a percentages 27% for example trimethylamine and salvadorine; fluorides and chlorides; adequate concentrations of sulfur, silica and vitamin C; and small amounts of, saponi, tannins, sterols and flavonoids [13,14] . Great quantities of potassium chloride and sodium chloride were showed , accompanied by other sulfur-containing organic substances (salvadorine and salvadourea) [15].

The Aim of Study

The present study efforts have been made to show the effect of hot aqueous and ethanol alcohol extracts of *S. persica* roots on the fertility of female albino rats. The following criteria have been tested:

- Weight of reproductive organs (ovary and uterus).
- Estimation of (follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2).
- Histological study (number of primary, secondary and Graafian follicles, ovarian diameter and endometrium thickness).
- Fertility test (number of embryos and pregnancy percentage).

MATERIALS AND METHODS

Laboratory Animals

The study includes (85) mature, healthy, fertile and adults female albino rats (weighting form 200-250g) with normal estrous cycle and age between (8-12) week were bred and maintained in the animal house of the faculty of veterinary medicine of Kufa university under control conditions of (12hr.) light and (12hr.) dark and temperature (24 ± 3°C). Animals were housed in plastic cages with free access pellets and water ad libitum and reproduce to obtained propitiate numbers and fertile males rats were used in fertility test.

Collection of Root

Miswak (*Salvadorapersica*) roots were collected from local market in Septembers 2014. The root were washed using tap water and twice by distilled washed ,then cut to approximately equal parts and immediately sprayed with (70%) ethanol to cause the enzymatic degradation and dried in oven at temperature (22%) for 24 hour . The dried roots were grinded properly using a mortar and pestle and grinder to obtained a powdered.

Hot Aqueous Extract

The method was prepared according to20 gm of powdered sample was suspended in 200 ml of distilled water. Extraction was done at 70˚C for 30 minutes, followed by filtering of the extracts using Whitman filter paper No.1. Extracts were then evaporated at 45˚C by rotary evaporator to form a paste, and further transferred into sterile bottles and refrigerated at 4˚C until use [16].

Ethanol Alcoholic Extract

The method was prepared according to Ethanol alcoholic extractwhich prepared by taking 20 gm of powdered sample that was extracted in soxholet by 200 ml of ethanol 95% solvent for 24 hr. then evaporated at 45˚C by rotary evaporator to form a paste, and further transferred into sterile bottles and refrigerated at 4˚C until used [17].

Vaginal Smears

The vaginal smears were taken by using sterile loop .The smears were transferred to glass slide and stained by methylene blue for 3-5 minutes than washed by distilled water and were examined under microscope for presence of certified epithelial cells [18].

Orally Treatment of Different Doses of hot Aqueous and Ethanol Alcohol Extracts of *Salvadorapersica* Roots

The intragastric application of both plant extracts were made by using female feeding needless (11,0 each) day and each female was received (1 ml) by (250 and 500) mg/kg of body weight in proestrus phase for aperiod (10 ,20 and 30)days.

Animals Sacrificing and Sample Collections

The female albino rats were dissected after a dose of ketamine and xylene . Anesthesia in a day eleven in a group of animal received both extracts for 10 days and in day twenty on in group of animals received both extras for 20 days and in treaty one day in a group received both extracts for 30 days after animal sacrificed both ovary and uterus were separated out from the abdominal adherent fats [19].

Blood Collection

Rats were anesthetized and the blood were taken by heart puncture through a midline incision above the abdominal hear the diagram.

Serum Sampling

Five milliliters of blood were taken by heart puncture ,the blood was allowed to clot in plain test tube at aspirated after centrifugation at 3000 rpm for 10 minutes ,divided into Eppendorf tubes and stores at -20°C until the measurement of the study parameters.

Hormonal assay

Estimation FSH hormone

The assay, rat Follicle Stimulating Hormone (FSH) hormone ELISA ,Kit was conducted according to the manufacturing company (Monobind INC. ,code number 425-300) that depended on the technique of the quantitative sandwich enzyme immunoassay ,its procedure explained in.

Estimation Luteinizing hormone

The assay, ratLuteinizing Hormone (LH) hormone ELISA ,Kit was conducted according to the manufacturing company (Monobind INC. ,code number 625-300) that depended on the technique of the quantitative sandwich enzyme immunoassay ,its procedure explained in.

Estimation Estradiol

The assay, ratEstradiol (E2) hormone ELISA ,Kit was conducted according to the manufacturing company (Monobind INC. ,code number 4925-300) that depended on the technique of the quantitative sandwich enzyme immunoassay ,its procedure explained in .

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Statistical Analysis

The results were expressed as(Mean ± Standard Deviation). Unpaired sample t-test was used for the comparison between the concentration and period and one way ANOVA test was used for the comparison among subdivided groups in the measured parameters were performed by using software packages Graph pad prism 6 (Version 6.01) for windows 2010, while the figures constructed using EXEL program of Microsoft Office 2010. P-value < 0.05 was used as a level of statistically significant [20].

RESULTS

Weight of ovaries and uterus

Effect ofinteractions of oral administration of differentsconcentrations of hot aqueous and ethanol alcohol extracts roots of *Salvadorapersica*on ovary and uterus weight

of female rats (organ weight in g / 100g of body weight).

The table (1) refers to the existence of a significant decrease (P < 0.05) in theweights of the ovaries after oral administration with hot aqueous (0.066±0.001and 0.049±0.003) g / 100g of body weight and ethanol alcohol (0.037±0.005 and 0.030±0.008) g / 100g of body weight extracts roots of *Salvadorapersica*for both concentrations (250 and 500) mg / kg respectively compared with the control group as it was (0.209±0.013) g / 100g of body weight and the concentration (500) mg / kg of ethanol alcohol extracts showed higher significant decrease in the weights of the ovaries compared withother concentrations of hot aqueous andethanol alcohol extracts .The results showed significant increase (P < 0.05) in the weights of the uteruses after oral administration with hot aqueous (0.917±0.037 and 1.294±0.144) g / 100g of body weightand ethanol alcohol (1.586±0.044 and 1.728±0.023)g / 100g of body weight extracts roots of *Salvadorapersica* for both concentrations (250 and 500) mg / kg respectively compared with the control group as it was (0.535±0.086) g / 100g of body weight and the concentration (500) mg / kg of ethanol alcohol extracts showed higher significant increase (P < 0.05) in the weights of the uteruses compared with other concentrations of hot aqueous and ethanol alcohol extracts (table 1). The alcoholic extracts displayed more significantly affected (P < 0.05) in decrease of ovaries weights and increases of uteruses weights compared with aqueous extracts (table 1).

Table (1): Effect ofinteractionsof oral administration of different concentrations of hot aqueous and ethanol alcohol extracts of roots of *Salvadorapersica*on ovary and uterus weight of female rats (organ weight in g / 100g of body weight).

Type of extract	Concentration mg / kg	Ovary weight (g /100g) Mean ± SD	Uterus weight (g /100g) Mean ± SD
Hot aqueous	250	0.066±0.001 (a)	0.917±0.037 (a)
	500	0.049±0.003 (a)	1.294±0.144 (b)
	Control	0.209±0.013 (b)	0.535±0.086 (c)
Ethanol alcohol	250	0.037±0.005 (c a)	1.586±0.044 (d)
	500	0.030±0.008 (d c)	1.728±0.023 (e d)
	Control	0.209±0.01(3e b)	0.535±0.086 (f c)

Interaction between different concentration and period of oral administration of hot aqueous and ethanol alcohol extracts roots of *Salvadorapersica*on weight of ovary and uterus of female rats (organ weight in g / 100g of body weight) .

From the results of figures (1 and 2) shows that significant decrease (P < 0.05) in the weights of the ovaries and significant increase (P < 0.05) in the weights of the uterus for both concentrations and periods of oral administration of two types of extracts in compared with the control group. The concentration (500) mg / kg of oral administration of ethanol alcohol extract roots of *Salvadorapersica*showed higher significant decrease (P < 0.05) in the weights of the ovaries and significant increase (P < 0.05) in the weights of the uterus compared with other concentrations of hot aqueous and ethanol alcohol extracts(figures 1 and 2). The period 30 days of oral administrationsof ethanol alcohol extract of *Salvadorapersica*root showed more

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significant effect (P < 0.05) in decrease of ovaries weights and increase of uterus weights compared with the other periods of oral administrations of hot aqueous and ethanol alcohol extracts (figures 1 and 2) . The ethanol alcohol extracts of *Salvadorapersica*roots showed more significant effect (P < 0.05) effect in decrease of ovaries weights and increase of uterus weights for both concentrations compared with hot aqueous extracts (figures 1 and 2).

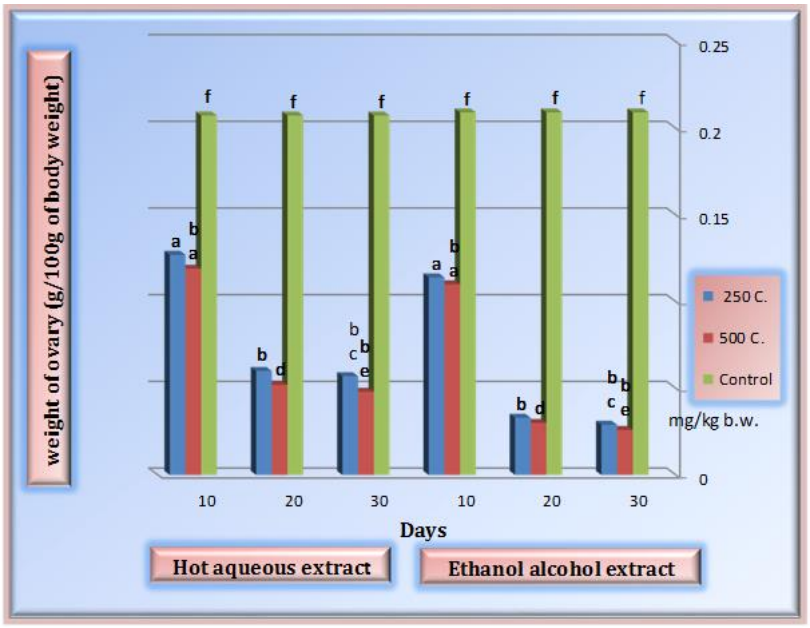

Figure (1): Interaction between different concentrationand period of oral administration of hotaqueous and ethanol alcohol extracts roots of*Salvadorapersica* on weight of ovary of femalerat (organ weight in g / 100g of body weight).

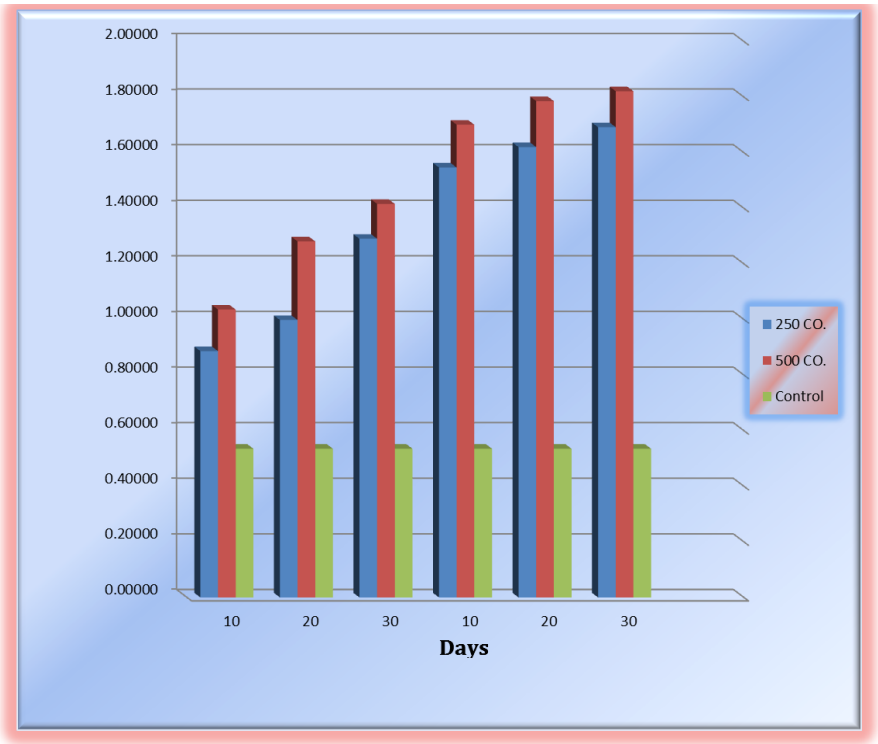

Figure (2): Interaction between different concentration and period of oral administration of hot aqueous and ethanol alcohol extracts roots of*Salvadorapersica*on weight of uterus of female rat (organ weight in g / 100g of body weight) .

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Hormonal profile

Effect of interactions of oral administration of differents concentrations of hot aqueous

The table (2) refers to the existence of a significant decrease ($P < 0.05$) in concentrations of follicle stimulating hormone (FSH)after oral administration with hot aqueous (1.347±0.106 and 0.783±0.177) mIU/ml andethanol alcohol (1.361±1.845 and 0.412±0.210) mIU/ml extracts roots of *Salvadorapersica*for both concentrations (250 and 500) mg / kg respectively compared with the control group as it was (6.574±0.483)mIU/ml and the concentration (500) mg / kg of ethanol alcohol extract showed higher significant decreases in the concentrations of follicle stimulating hormone (FSH) compared with other concentrations of hot aqueous and ethanol alcohol extracts. The results showed significant decrease ($P < 0.05$)) in concentrations of luteinizing hormone (LH) after oral administration with hot aqueous (2.913±0.953 and 1.764±0.091) mIU/ml and ethanol alcohol (2.925±0.769 and 0.709±0.146) mIU/L extracts roots of *Salvadorapersica*for both concentrations (250 and 500) mg / kg respectively compared with the control group as it was (5.580±0.354) mIU/ml and the concentration (500) mg / kg of ethanol alcohol extract showed higher significantdecrease ($P < 0.05$) in the concentrations of luteinizing hormone (LH) compared with other concentrations of aqueous and alcoholic extracts (table 2). The table (2) refers to the existence of a significant increase ($P < 0.05$) in concentrations of estradiol hormone after oral administration with hot aqueous (58.905±4.674 and 73.178±4.033)pg/ml and ethanol alcoholic (83.683±2.663 and 90.709±3.389) pg/ml extracts roots of *Salvadorapersica*for both concentrations (250 and 500) mg / kg respectively compared with the control group as it was (43.420±1.688) pg/ml and the concentration (500) mg / kg of ethanol alcohol extract showed higher significant increases in the concentrations of estradiol hormone ng/ml compared with other concentrations of hot aqueous and ethanol alcohol extracts.

Table (2): Effect ofinteractions of different concentrations of hotaqueous and ethanol alcohol extracts of roots of*Salvadorapersica* on some hormonal levels.

Type of extract	Concentration mg / kg	Hormonal parameters		
		FSH mIU/ml Mean ± SD	LH (mIU/ml) Mean ± SD	Estradiol (pg/ml) Mean ± SD
Hot aqueous	250	1.347±0.106 (a)	2.913±0.953(a)	58.905±4.674(a)
	500	0.783±0.177(a)	1.764±0.091(a)	73.178±4.033(b)
	Control	6.574±0.483(b)	5.580±0.354(b)	43.320±1.688 (c)
Ethanol alcohol	250	1.361±1.845(a)	2.925±0.769(a)	83.683±2.663(d)
	500	0.412±0.210(a)	0.709±0.146 (ca)	90.709±3.389(ed)
	Control	6.574±0.483(cb)	5.580±0.354(db)	43.320±1.688 (fc)

Interaction between different concentration and period of oral administration of hot aqueous and ethanol alcohol extracts roots of *Salvadorapersica*on some hormonal levels.

From the results of figures (3,4 and 5) shows that significant decreases ($P < 0.05$) in concentrations of both follicle stimulating hormone (FSH) andluteinizing hormone (LH) and significant increases ($P < 0.05$)

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concentrations of estradiol hormone for both concentrations and periods of oral administration of two types of extracts in compared with the control group.The concentration (500) mg / kg of oral administration of ethanol alcohol extracts roots of *Salvadorapesica*showed higher significant decrease ($P < 0.05$) in concentrations of both follicle stimulating hormone (FSH) and luteinizing hormone (LH) and significant increase ($P < 0.05$) concentrations of estradiol hormone compared with other concentrations of hot aqueous and ethanol alcohol extracts (figures 3, 4 and 5).

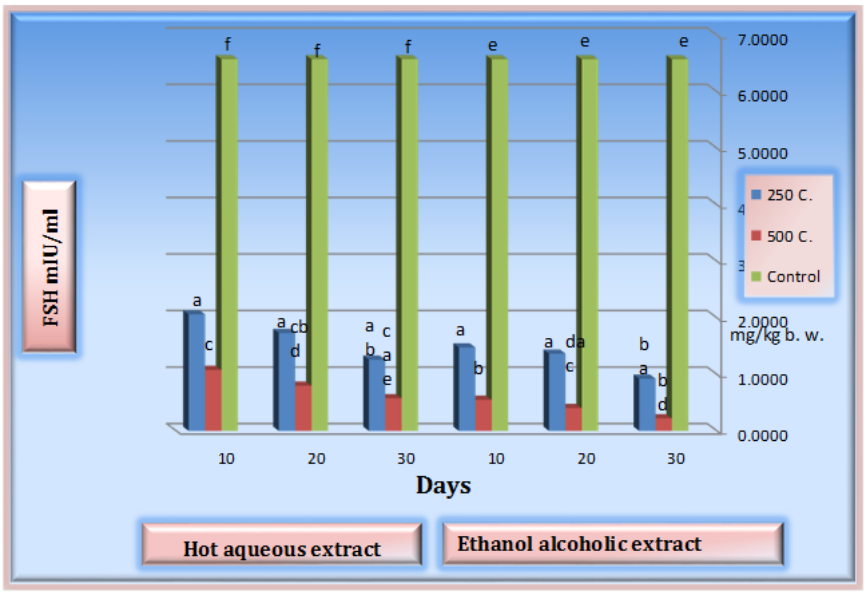

Figure (3): Interaction between different concentration and period of oral administration of hot aqueous and ethanol alcohol extractsroots of *Salvadorapersica*on follicle stimulating hormone (FSH), levels.

The period 30 days of oral administrations showed more significant effect ($P < 0.05$) in decrease of concentrations for both follicle stimulating hormone (FSH) and luteinizing hormone (LH) and increase of concentrations of estradiol hormone compared with other periods of oral administrations of hot aqueous and ethanol alcohol extracts (figures 3,4 and 5) .

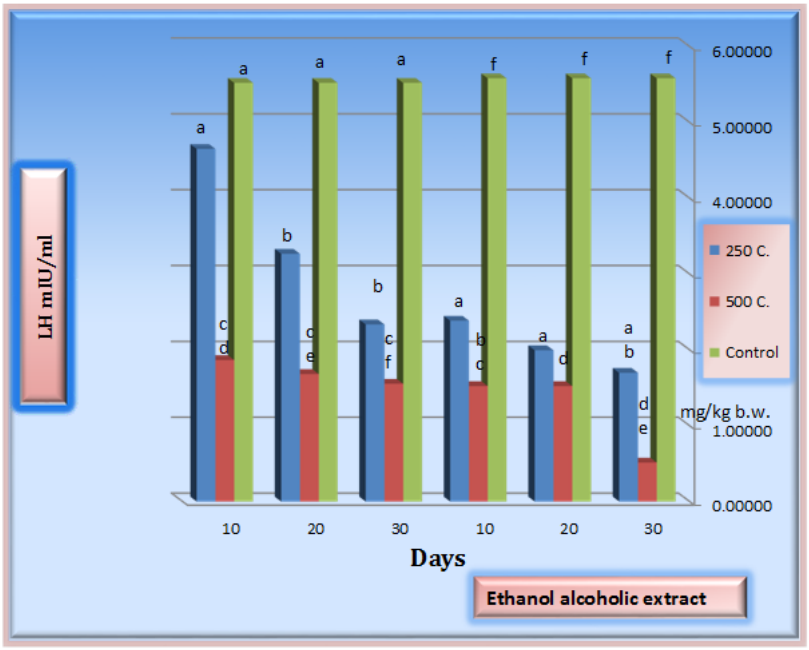

Figure (4):Interaction between different concentration and period of oral administration of hot aqueous and ethanol alcohol extracts roots of *Salvadorapersica*on luteinizing hormone (LH) levels.

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The ethanol alcohol extracts of *Salvadorapersica*roots showed more significant effect ($P < 0.05$) in decrease concentrations for both follicle stimulating hormone (FSH) and luteinizing hormone (LH) and increase of concentrations of estradiol hormone compared with hot aqueous extract (figures 3, 4 and 5).

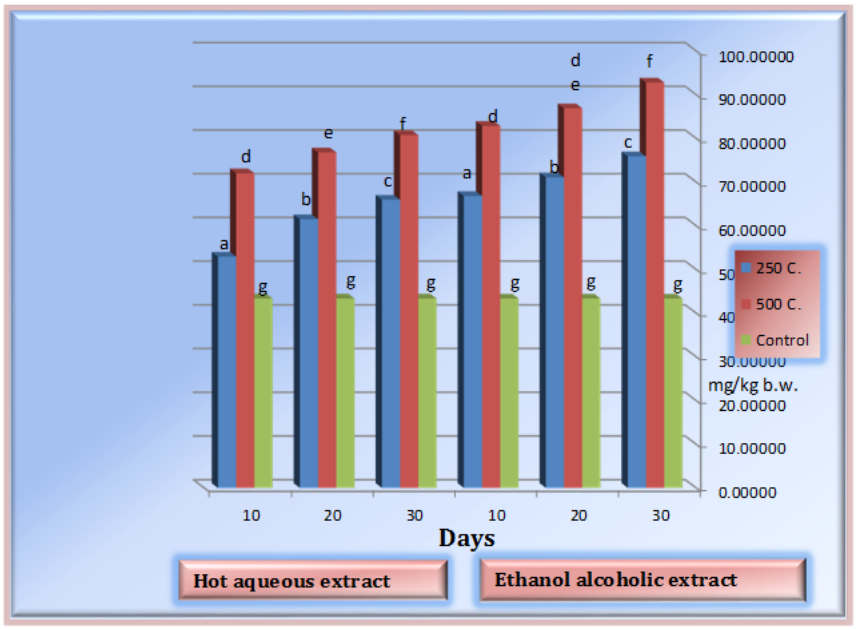

Figure (5): Interaction between different concentration and period of oral administration of hot aqueous and ethanol alcohol extracts roots of *Salvadorapersica*on Estradiol hormone levels.

DISCUSSION

Effect of Hot Aqueous and Ethanol Alcohol Extracts of*Salvadorapersica* on Ovary and Uterus Weight

The current results revealed a significant decrease ($p<0.05$) in ovarian weight after oral administration by (250 and 500) mg/kg of hot aqueous and ethanol extract in comparison with control group.The study of [21] demonstrated that Miswak has a significant decrease ($p<0.05$) in the relative weight of ovary and this may due to reduction in FSH level which is responsible for follicles growth . The present results agree with many studies that indicate a significant roles of many other plants ,on ovarian weight and documented that FSH level reduction may play important roles in decrease of ovarian weight and follicle development [2,5,22,6]. Previous study suggested that decrease in ovarian weight may be resulted from glycogen content reduction therefore decrease availability of ovary for estrogen [17]. Plant extract *Salvadorapersica* may play important roles on hypophysial-pituitary –ovarian axis and inhibition of this axis reduced the secretion of gonadotropin-releasing hormones from hypothalamus and FSH,LH from anterior pituitary gland . The present study proved a significant increase ($p<0.05$) in uterus weight after administration by both extracts (250 and 500) mg/kg of aqueous and ethanol extract of *Salvadorapersica* . Estrogen and progesterone hormones have a significant role on uterine growth development and lead to increase uterus weight ,therefore the estrogenic activity of *Salvadorapersica*may lead to increase uterine weight [21]. The present study agrees with other studies that used different medicinal plants and have a phytoestrogen activity such as terpeniods , phenolic and alkaloid compound which lead to increase estrogen level and uterine weight [23,24,25,5,6]. The results of present study showed that ethanol extracts were more active than aqueous extracts, former studies suggested that separation of more active compound by ethanol extract such as alkaloid ,phenols , flavonoids, essentials oils, steroids tannins and terpeniods in comparing with hot aqueous extract [26,27]. The significant effect after long duration periods on ovarian and uterine weight resulted from decrease the activity of hypophysial-pituitary axis and lead to decrease FSH and LH secretion from anterior pituitary gland . Research by [32] showed that there is a significant Correlation between Cardiovascular Diseases in Obese Men with The Inflammatory Markers Dyslipidemia, C-Reactive Protein and Tumor Necrosis Factor-α. Further studies by Aldujali and colleagues [33,34,35] showed significant relationship Between Adipocyte Fatty Acid–Binding Protein In Obese Men With Cardiovascular Diseases and the Effect of Methanolic Leaf Extract of *Moringa oleiferaon* some Biochemical Markers in obesity induced rats. On the other hand they conducted a research

between the effect of Methanolic Extract of *Moringa oleifera* and Exogenous Ghrelin on Lipid Profile in Atherogenic Rats and have found a significant impact of moringa extract on the reduction of lipid profiles in Atherogenic Rats [35].

Efect of Hot Aqueous and Ethanol Alcohol Extracts of *Salvadorapersica* on Hormonal Level

FSH and LH levels

The present study indicates a significant decrease ($p<0.05$) in FSH and LH level after oral administration by (250 and 500)mg/kg of hot aqueous and ethanol alcohol extracts compared with control groups . The significant decrease of FSH and LH level due to increase level of prolactin hormone which is produced by anterior pituitary gland ,hyperprolactinmea lead to decrease the level of FSH and LH from anterior pituitary gland by inhibitory effect on hypophysial-pituitary – axis and reduction in both hormones lead to inhibition of both follicles development and ovulation [28,17].The current study agrees with some studies that indicate an inhibitory effects of some medicinal plants especially on hypophysial-pituitary – axis [2,29,5,6]. Many compounds have been isolated from ethanol extracts *Salvadorapersica*roots such as alkaloids phenols terpeniods ,flavonoids and tannins have more potent role on pituitary gland to decrease FSH and LH secret [30].[22]suggested that salvadorire and salvadourea compounds of *Salvadorapersica*have indirect roles on both ovaries and pituitary gland. The present study indicated a significant increase in estradiol level . Estrogenic activity of *Salvadorapersica*extracts may lead to reduction in ovarian growth as result of negative feedback mechanism on hypothalamus or hypothalamus -pituitary - axis and reduction in both FSH and LH level [11]. This study revealed that ethanol extract of *Salvadorapersica*plant have more effective than hot aqueous extract . [6] indicated more compounds are separate from ethanol alcohol extracts such as alkaloids ,phenols ,volatile oil ,flavonoids and tannins than hot aqueous . The high doses of *Salvadorapersica*extract 500 mg/kg and increase of duration lead more decrease in FSH and LH level many studies proved that high doses of *Salvadorapersica*with long duration have more active decrement of FSH and LH level [26,27].

Estradiol level

The current results demonstrated a significant increase ($p<0.05$) in estradiol level after oral administrated of both extracts (250 and 500) mg/kg of the hot aqueous and alcoholic extracts of *Salvadorapersica* roots. Current results agree with many researchers indicated that phytoestrogen tend to higher affinity to bind with estrogen receptor α and β lead to increase in estrogen level [31,32,27,6]. This study disagrees with study of[51] that suggested a decrease in estradiol level after administration of 50 and 100 mg /kg of *Salvadorapersica*roots . The increase level of estradiol level may be discussed as increase in estrus and proestrus phase duration . Estrogenic compound may be present in the roots such as alkaloid ,phenolsterpeniods ,tannins ,volatile oil and other and these compound have an estrogenic activity.

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