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Phytoestrogen Effect of *Ambrosia maritima* (Damsissa) on the Ovarian Activity of Immature Rabbits

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Abstract: Damsissa ethanol extract was prepared and dissolved in tween 80 and distilled. Water to obtain 20% suspension for use. Phytochemical screening was carried out according to the prescribed methods. Biological assays were done for detection of estrogen-like activity of the extract using 28 immature female mice (10-13g b. wt.) divided into 4 groups (n=7), the first group was given olive oil(Control group) s/c in a dose of 0.1ml/kg b.wt. for 7days.The 2nd group (Standard group) was given estradiol monobenzoate daily by s/c injection in a dose of 0.4ug/kg b.wt. for 7 days. The 3rd and the 4th groups were treated with damsissa ethanol extract orally in a dose of 5 and 10mg/10 g, b. wt., respectively, daily for 7days. At the end of experiment the mice were weighed then they were sacrificed and their uteri were dissected and weighed. *In vivo* study was carried out on virgin rabbits. Thirty immature rabbits (1-1.2 kg b. wt.) were divided into three groups (10 rabbits/group). The first group was given 0.1 ml/ kg. b.wt. olive oil subcutaneously daily for 30 days (Control group). The second group (Standard group) was given 0.4 µg / kg. b.wt. oestradiol monobenzoate daily by s/c administration for 30 days. The third group was given 800mg/kg. b.wt. damsissa ethanol extract orally for the same period. The blood samples were taken from the ear vein before treatment and at the end of experimental period blood samples were used for determination of hemogram and serum samples for hormonal assay. The animals were slaughtered at 30th days of experiment and the ovaries and uteri were dissected out and weighed. The oocytes were collected from the ovarian follicles and cultured *in vitro* for maturation under conditioned environment for 24 hrs. Phytochemical screening of active constituents of damssisa ethanol extract contain carbohydrates and / or glycosides, flavonoids, tannins, steroids and / or triterpenoids, alkaloids and resins. The tested extract produced significant oestrogen-like activity with various degrees according to the given dose, with lower significant ($p < 0.05$) in a dose of 50mg/100gm b.wt. and higher significant ($p < 0.01$) in a dose of 100mg/100gm b.wt. In rabbits, damssisa (400mg/kg.b.wt.) produced significant oestrogen-like activities ($p<0.05$) in the form of increased both uterine and ovarian weights as compared to the control group. Histopathological examination of the Ovaries and uteri of rabbits from group treated with damsissa extract revealed degenerated corpora lutea and interstitial fibrosis in the ovaries and congestion of stromal blood capillaries in the uterus.

Key words: Damsissa · Phytochemical · Ovary · Oestrogen · Histopathology - Rabbits

INTRODUCTION

Ambrosia maritima (Damsissa) is a plant distributed locally on the Nile delta, on muddy canal banks, Oases and Mediterranean region. And regionally in Egypt, Mediterranean Costal strip from El Sallum to Rafah. The plant grows on the Nile and canal banks on the sediments from the Nile flood. After the construction of the high Dam and the lack of these sediments, the plant became rare. This threatens this species in addition to the continuous collection for folk medicinal uses. It was found that Damssisa contains flavonoids, coumestans and isoflavons as other phytoestrogens.

Phytoestrogens are defined as any plant substance or metabolite that induces biological responses in vertebrates and can mimic or modulate the actions of

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endogenous oestrogen by binding to oestrogen receptors. The majority of phytoestrogens belong to a large group of substituted phenolic compounds known as flavonoids. Three classes of flavonoids, isoflavones, coumestans and prenylated flavonoids are phytoestrogens that possess the most potent oestrogenic activity. Knight and Eden [1] divided phytoestrogens into 3 major categories: isoflavones, lignans (Precursors and naturally occurring) and coumestans. Scientific interest in phytoestrogens increased in the 1940's when sheep that grazed on subterranean clover (*Trifolium subterraneum, L.*), which contains high concentrations of phytoestrogens, were found to have reproductive dysfunctions like cystic ovary, very low lambing rates, prolapse of the uterus and dystocia, Severe metritis, pyometron and *hydrops uteri* were also observed [2]. The main known phytoestrogens isoflavones daidzein, genistein, formononetin and biochanin A. Phytoestrogens can induce both oestrogen agonistic and antagonistic effects, depending on the tissue, oestrogen receptor content and endogenous levels of oestrogen. The oestrogenicity of phytoestrogrns coumestans is approximately 1/1,000 and of isoflavones approximately l/ 10,000, relative to the activity of oestradiol-17beta. Phytoestrogens mimic the actions of oestradiol, but their effects are not necessarily identical; for example, there are no reliable reports of phytoestrogens causing behavioral estrus. Phytoestrogens may also have antiestrogenic activity. Folman and Pope [3] indicated that phytoestrogens compete with endogenous steroids, so that the balance between oestrogenic and antiestrogenic activity is determined by the ratio of phytoestrogens to oestrogen. This may explain why oestrogenic effects predominate in the sheep, but antiestrogenic effects are mainly reported in humans, in which circulating concentrations of steroidal oestrogen are relatively high [4]. Cattle have relatively low circulating concentrations of oestradiol, so it may be expected that oestrogenic effects would dominate in this species. Dietary phytoestrogens are promoted as alternatives to synthetic oestrogen for hormone replacement therapy. Recent studies revealed that isoflavones binding affinity for ERb is significantly higher than for ERa [5,6]. However, individual isoflavones exhibit different affinity for each of the two ER isoforms [7]. Moreover, chronic exposure to dietary isoflavones may change ER expression in reproductive tissues including ovaries [8,9].

The present study aimed to evaluate the pharmacological effect of damsissa ethanol extracts on the ovarian activity of immature female rabbits as well as to determine the active principles of this plant and to examine their oestrogen like activities.

MATERIALS AND METHODS

In the present study, the preliminary photochemical determination of active constituents the tested plant (Damsissa) was carried out. The oestrogen like effect of the tested plant ethanol extract was performed using uterine weight test in immature mice. In addition, the effects of damsissa extract on ovarian activity in immature rabbits were determined using ovarian and uterine weights and *in vitro* maturation (IVM) and fertilization (IVF) of oocytes as well as serum levels of oestrogen and progesterone. Histopathological examination of ovary and uterus were also performed in this study.

Materials

Plant: The tested plant *(Ambrosia maritima,* Family *Compisitae*) was purchased from a local market of Medicinal Plants and Herbs, Cairo, Egypt. The plant was air-dried, pulverized and kept in tightly closed glass container at room temperature till subjected to preliminary phytochemical investigation.

Animals

Mice: Immature female mice (10-13 g body weight) were used for revealing the estrogen like effect of the tested plant extract. Mice were purchased from the Animal House, National Research Center, Dokki, Egypt.

Rabbits: Immature female rabbits weighing 1-1.2 kg body weight were used for revealing the effect of the tested extract on ovarian activity, serum levels of estrogen and progesterone and histopathology of the uterus and ovaries.

Methods

Preparation of Plant Ethanol Extract: To each 250 gram of dried plant powder, one litter of ethyl alcohol 95 % was added in a wide mouth plastic container and kept for a night. On the next morning filtration of the container content was carried out using double layer gauze. The liquid ethanol extract was evaporated in a Rota vapor apparatus at 40°C under reduced pressure for drying till complete evaporation of alcohol. The obtained extracts were dissolved in Tween 80 and distilled water to obtain 20% suspension for use.

Phytochemical Screening of Active Principles of the Plant: The tested plant (*Ambrosia maritima*) was subjected to the phyto-chemical tests as explained by Claus [10] as follows; tests for carbohydrates and / or glycosides (Molish`s test, Fehling's test and Benedict's test), tests for tannins, tests for alkaloids and/ or nitrogenous bases as described by Smolenski *et al.* [11], tests for Flavonoids, tests for saponins as explained by Dafert *et al.* [12], tests for unsaturated sterols and / or tri-terpenes and tests for Resins.

Oestrogen like Effects in Mice: Twenty eight immature mice weighing 10-13 grams body weight were divided into four equal groups (n = 7). The first group was given olive oil (Control group) by subcutaneous administration in a volume of 0.1ml/ 100g.b.wt./daily for 7 days. The second group (Standard group) was given oestradiol monobenzoate daily by subcutaneous administration in a dose of 0.1 µg / 100g.b.wt. for 7 days. The third and fourth groups were given damsissa ethanol extract by oral administration in a dose of 50and 100mg/100 g.b.wt. respectively, daily for 7 days. At the end of experiment the mice were weighed on digital balance then they were sacrificed and their uteri were dissected and weighed.

Rabbits and Husbandry: Thirty immature (1-1.2 kg) New Zealand White (NZW) female rabbits were purchased from the same herd in a commercial farm, for the purpose of this study. They were individually housed in metal wire mesh cages provided with separate facilities for feeding and water supply.

General Layout of Experiment: The experiment was carried out at the experimental rabbit try of lab animal house of National Research Center, Dokki, Giza-Egypt. Rabbits were left two weeks for acclimatization before treatment. Blood samples (Heparinized and serum) were collected at slaughter time. They offered a commercial balanced ration pellets (Atmida Co.).

Effect of the Tested Plant Extract on Ovarian Activity of Rabbits: Thirty immature rabbits weighing 1-1.2 kg body weight were divided into three groups (10 rabbits in each group). The first group was given olive oil by subcutaneous administration in a volume of 1ml/ kg.b.wt. daily for 30 days and kept as a control group. The second group (Standard group) was given oestradiol monobenzoate daily by subcutaneous administration in a dose of 10µg / kg.b.wt. for 30 days. The third group was given damsissa ethanol extract by oral administration in a dose of 400mg/kg.b.wt. daily for the same period. The animals were slaughtered at 30th day of experiment and the ovaries and uteri were dissected out and weighed. All uteri from the treated group were preserved in 10% neutral formalin solution till the histopathological examination. The dissected ovaries from each group were divided into two halves; the first half was preserved in 10% neutral formalin solution for histopathological examination and the other half was used for collection of follicular oocytes for *in vitro* maturation (IVM) and fertilization (IVF) of oocytes. Blood samples were used to estimate oestrogen and progesterone levels in the serum.

Blood Collection and Analysis: Blood samples were collected at the slaughter time. Two blood samples were collected from each animal, the first one (0.5 ml) was collected on heparinized vacuum tube for hemogram. The second (3.0 ml) was centrifuged for serum separation and stored at -80°C until further biochemical analysis.

***In Vitro* Maturation and Fertilization of Rabbit Follicular Oocytes**

Ovaries: The ovaries were collected within 20 minutes after slaughtering of the rabbits and kept in physiological saline (0.9% NaC1) containing antibiotics (100 µg/ml Streptomycin and 100 I.U./ml Penicillin) at 32-35°C. The ovaries were transported from Animal House to the laboratory within 30 minutes after slaughtering [13]. The ovaries in the lab. were rinsed several times in normal saline then transferred to a glass Petri dish containing 5 ml of modified phosphate buffer saline (M-PBS).

Oocytes Collection: The ovaries were sliced into small species and the ovarian follicles were punctured using sterilized syringe needle. The oocytes were picked up using prepared sterilized glass Pasteur pipette with suitable pore thin washed three times in M-PBS, examined under stereomicroscope for classification and evaluation.

Oocyte Classification (Grades): According to Leibfried and First [14] the oocytes were classified under the stereomicroscope based on the number of layers of the cumulus cells surrounding the oocyte into three categories:

Grade 1: Oocyte with complete compact dense cumulus oophrus more than three layers.

Grade 2: Oocyte with compact cumulus layer not completely surrounding the oocyte.

Grade 3: Oocyte enclosed only by the zona pellucida without cellular investment.

Only the oocytes surrounded by complete compact layers of cumulus cells (Grade 1) were used for maturation and fertilization.

***In vitro* Maturation of Oocytes:** The selected oocytes were washed three times in maturation medium TCM-199 (pH 7.2-7.4) supplemented with fetal calf serum and antibiotics. The oocytes (10-20 oocytes) were placed in 50µl-100µl droplet of maturation medium in a four- wheel culture plate (Nunclon, Denmark), covered with sterilized mineral oil (Sigma, USA).The culture dishes were placed in a CO_2 incubator (95% maximum humidity, 5% CO_2 at 38.5°C) for 24 hrs.

Assessment of Maturation: After 24 hrs, maturation rate was assessed according to the degree of cumulus cells expansion [15].

***In vitro* Fertilization:** Preparation of semen for *in vitro* fertilization (Sperm capacitation):The procedure was carried out according to the method described by Niwa and Ohgoda [16] and Irritani and Niwa [17].

***In vitro* Fertilization Procedure:** *In vitro* matured oocytes (5-10) were transferred to the sperm micro droplet which was already prepared. Spermatozoa and oocytes were co-cultured for five hours in CO_2 incubator at 38.5°C under 5 % CO_2 and 95% maximum humidity. The oocytes were washed three times with culture media (TCM-199) supplemented with 10% FCS and 1µl/ml (40mg/ml antibiotic-antimycotic gentamicin), then transferred to 50 µl droplets of the same medium and cultured for 6 days at 38.5°C in CO_2 incubator (5% CO_2 and 95% maximum humidity). After 48 h of insemination, the oocytes were then examined for fertilization as indicated by cleavage rate. The criterion for fertilization was cleavage to two-to four- cell stage 48 hours after insemination [18].

Determination of Oestrogen and Progesterone Hormones: Oestrogen and progesterone hormones were estimated by radioimmunoassay technique (RIA) using oestrogen and progesterone kits according to the method described by Xing *et al.* [19]. These kits were purchased from Siemens Medical Solution Diagnostics, Germany.

Histopathological Examination: At the end of the experiment, rabbits were sacrificed and the tissue samples including uterus and ovary were taken and subjected for microscopical examination according to Luna [20].

Statistical Analysis: Statistical analysis was carried out by using one way ANOVA followed by Duncan test and SPSS version 9.0 the difference of means at $p < 0.05$ considered significantly [21].

RESULTS

The current study was performed to evaluate the effects damsissa ethanol extract on ovarian activity as estimated by ovarian weight, *in vitro* maturation and fertilization rates of the collected oocytes, serum level of oestrogen and the histopathological examination of ovarian tissue of immature rabbits. Oestrogen like effect of this plant extract in immature mice was also examined. In addition, phytochemical screening for the active principles of the plant extract was carried out. The obtained results are illustrated in Tables (1-6) and Figures (1-6).

Preliminary phytochemical screening of active constituents of the tested plant (Damsissa) revealed that it contains carbohydrates, flavonoids, tannins, triterpenoids, alkaloids and resins. Concerning saponins, damsissa was devoid of this active constituent as recorded in Table 1.

The effect of damsissa ethanol extract on body weight, uterine weight in immature mice was recorded in Table 2. The results revealed that subcutaneous administration of oestradiol monobenzoate (Standard group) in a dose of 0.1 µg / 100gm.b.wt. caused significant ($p < 0.01$) increase in uterine weight of immature mice. This increase reached to 108.75 ± 7.88 (mg) compared to 65.00 ± 5.00 (mg) of the control group (Olive oil). The tested extract produced significant oestrogen-like activity with various degrees according to the given dose. With lower significant ($p < 0.05$) in a dose of 50mg/100gm b.wt. and higher significant ($p < 0.01$) in a dose of 100mg/100gm b.wt. Oral administration of damsissa ethanol extract in a dose of 50mg/100gm.b.wt. to immature mice resulted in significant ($p < 0.05$) increase in uterine weight that reached 72.25 ± 5.15 (mg) compared to 65.00 ± 5.00 (mg) of the control group (Olive oil). Damsissa ethanol extract when given orally in a dose of 100mg/100gmb.wt. to immature mice showed significant ($p < 0.01$) increase in uterine weight to 80.11 ± 6.18 (mg) as compared to 65.00 ± 5.00 (mg) of the control group.

Table 1: Preliminary phytochemical screening of active constituents of Damsissa ethanol extract

	Extracts	
Damsissa extract	Tests	
+ve	Molish's test	Carbohydrate and / or glycosides
+ve	Fehling's test	
+ve	Benedict's test	
+ve	Shinoda's test	Flavonoids
+ve	Ferric chloride test	Tannins
+ve	Phenazone test	
+ve	Liebermann- Burchard test	
+ve	Salkowisk test	Sterols and / or triterpenes
+ve	Mayer's R.	Alkaloids
+ve	Dragendorff's R.	
+ve	Wagner's R.	
-ve	Froth test	Saponins
-ve	Haemolysis test	
+ve	Turbidity test	Resins

R= Reagent +ve means positive -ve means negative

Table 2: Effect of oral administration of Damsissa ethanol extract at two dosage levels on the uterine weight of immature mice. (n = 7)

Groups and doses	Body weight (gm)	Uterine weight (mg)
Olive oil (0.1 ml/ 100gm/ b.wt.)	12.11 ± 0.18	65.00 ± 5.00
Oestradiol (0.1 µg /100gm.b.wt.)	11.73 ± 0.12	108.75 ± 7.88**
Damsissa (50mg / 100gm b.wt.)	11.45 ± 0.12	72.25 ± 5.15*
Damsissa (100mg / 100gm b.wt.)	11.70 ± 0.18	80.11 ± 6.18**

Data are expressed as mean ± SE

*Significant at $p < 0.05$ **Significant at $p < 0.01$

Table 3: Effect of oral administration of damsissa ethanol extract to immature rabbits for 30 days on body weight, uterine weight and ovarian weight. (n = 5)

Groups	Body weight (kg)	Uterine weight (g)	Ovarian weight (g)
Control (olive oil, 1 ml/ kg. b.wt.)	2.38 ± 0.25	3.16 ± 0.09	0.14 ± 0.04
Standard (Oestradiol, 10µg / kg.b.wt.)	2.42 ± 0.14	7.02 ± 0.65***	0.30 ± 0.01***
Damsissa (400 mg / kg.b.wt.)	2.36 ± 0.20	5.30 ± 0.88*	0.24 ± 0.03*

Data are expressed as mean ± SE

* Significant at $p < 0.05$ *** Significant at $p < 0.001$

Table 4: Effect of oral administration Damsissa ethanol extract to Rabbits for 30 days on follicular dynamics and recovery rate in immature rabbits. (n = 5)

Follicular size									
Small		Medium		Large					
%	No.	%	No.	%	No.				
11.0	66	69.0	414	20.0	120	60.0	600	10	Olive oil (1ml/kg.b.wt.)
6.7	64	42.3	406	51.0	490	96.0	960	10	Oestradiol (10 µg /kg. b. wt.)
15.5	112	52.0	374	32.5	234	72.0	720	10	Damsissa (400mg/ kg. b. wt.)

Table 5: Effect of oral administration of damsissa ethanol extract to immature rabbits for 30 days on quality of recovered oocytes, maturation and fertilization rates *in vitro*

	Quality of oocytes (%)				
	Grade I	Grade II	Grade III		
Olive oil (1ml /kg. b. wt.)	70.0	25.0	5.0	80.0	45.0
Oestradiol (10 µg /kg. b. wt.)	65.0	20.0	15.0	82.0	40.0
Damsissa (400mg / kg. b. wt.)	45.0	20.0	35.0	45.0	15.0

Table 6: Effect of oral administration of Damsissa ethanol extracts to immature rabbits for 30 days on serum levels of oestrogen and progesterone in immature rabbits. n = (10)

Progesterone (ng/ml)	Oestrogen (pg/ml)	Group
0.6 ± 0.1	15.5 ± 0.3	Control (olive oil 1 ml/ kg. b.wt.)
0.6 ± 0.3	47.2 ± 0.6***	Oestradiol (10µg / kg.b.wt.)
0.6 ± 0.1	30.3 ± 0.2***	Damsissa (400 mg / kg. b.wt.)

Data are expressed as mean ± SE *** Significant at p < 0.001

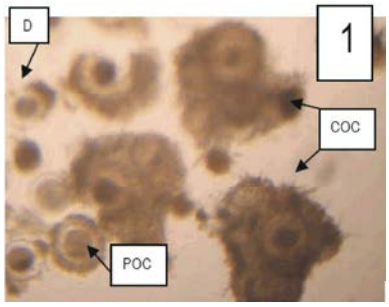

Fig. 1: Quality of recovered rabbit oocytes, excellent(COC), good(POC) and fair (D)grades after treatment with plant extracts for 30 days

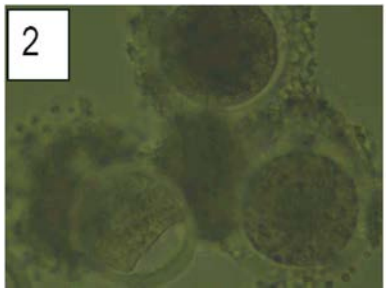

Fig. 2: Rabbit oocytes after maturation *in vitro* for 24hr under Conditioned environment, showing expansion of granuolosa cell layers (X 400)

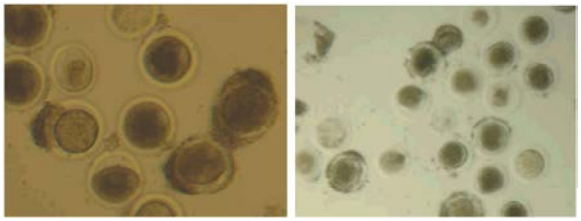

Fig. 3: Showing different forms of degenerated, retarded growth, shrinkage or granulated cytoplasm of recovered oocytes after treatment with the tested plant extracts

The effect of tested ethanol extracts on body weight, uterine weight and ovarian weight in immature rabbits was recorded in Table 3. The results revealed that subcutaneous administration of oestradiol monobenzoate (Standard group) in a dose of 10µg / kg.b.wt. caused significant ($p < 0.001$) increases in both uterine and ovarian weights of immature rabbits. These increases reached to 7.02 ± 0.65 and 0.30 ± 0.01 (g) compared to 3.16 ± 0.09 and 0.14 ± 0.04 (g) of the control group, respectively. The tested extract of damsissa produced significant oestrogen-like activity with various degrees. Oral administration of damsissa ethanol extract produced significant ($p < 0.05$) oestrogen-like activity. The increases caused by damsissa extract in both uterine and ovarian weights were 5.30 ± 0.88 and 0.24 ± 0.03 (g) compared to 3.16 ± 0.09 and 0.14 ± 0.04 (g) of the control group, respectively, as shown in Table 3.

As shown in Table 4 the recovery rate of oocytes / ovary of immature rabbits given either olive oil (Control group) or oestradiol monobenzoate (Standard group) for 30 days was 60 or 96, respectively. In immature rabbits given orally damsissa ethanol extract, the recovery

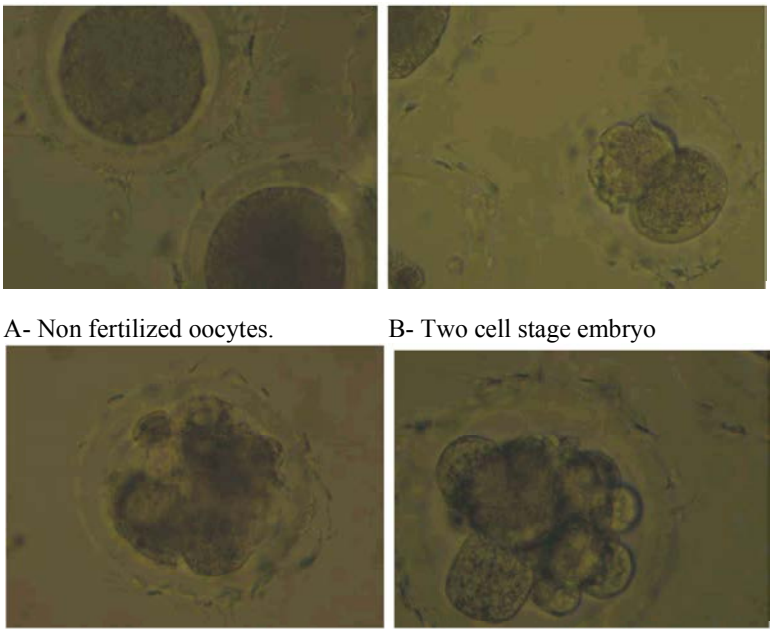

Fig. 4: Oocytes/embryos at different developmental stages showing abnormalities in growth or poor quality embryos:
A- Non fertilized oocytes.
B- Two cell stage embryo
C- 4-8 cell stage embryo (Degenerated).
D- Morula stage embryo (Some cells degenerated)

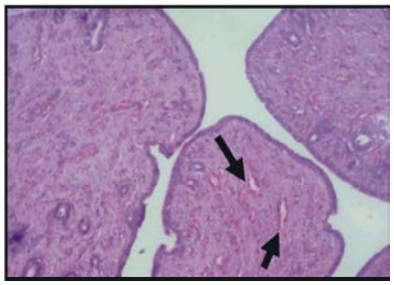

Fig. 5: Uterine section of a rabbit given orally damsissa ethanol extract in a dose of 400 mg / gm.b.wt. showing congestion of stromal blood capillaries. (H & E x 100)

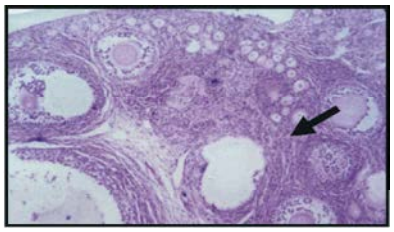

Fig. 6: Ovarian section of a normal rabbit given olive oil (1 ml / kg.b.wt.) showing normal follicles in different stages of development. (H & E x 100)

rate was 72 oocyte/ovary. Concerning the follicular size, the percentages of large follicular oocytes were 20%, 51% and 32.5% for olive oil, oestradiol monobenzoate and damsissa extract, respectively. The percentages of medium follicular oocytes were 69%, 42.3% and 52% for olive oil, oestradiol monobenzoate and damsissa extract, respectively. The percentages of small follicular oocytes were 11%, 6.7and 15.5% for olive oil, oestradiol monobenzoate and damsissa extract, respectively.

The quality, maturation and fertilization rates of rabbit oocytes *in vitro* of different groups are shown in Table 5 and demonstrated in Figs (1-4). The quality of oocytes varied according to the treated group in comparison with control one. Subcutaneous administration of oestradiol monobenzoate (10 µg/kg b.wt.) not affected significantly on the grades I and II of oocytes as it averaged 65% and 20% compared to 70% and 25% of the control group (Olive oil), respectively. Damsissa ethanol extract (400 mg/kg b.wt.) decreased the quality of oocytes specially the oocytes of grade I (45% compared to the control group (70%). Grade II oocytes not greatly varied among treated group, it averaged 20% in both treated group given oestradiol monobenzoate and damsissa

ethanol extract, in comparison with control group (25%). Plant extract recorded a worst effect on the oocytes quality, where the bad quality oocytes (Grade III) reached 35% in the treated group compared to 5% of the control group. *In vitro* maturation rate of rabbit oocytes decreased in the treated group given (45%) compared to 80% of the control group. In addition, the fertilization rates were decreased to 15% compared to 45% of the control group.

The effect of the tested extract on serum oestrogen and progesterone levels was recorded in Table 6. The obtained results revealed that subcutaneous administration of oestradiol monobenzoate (Standard group) in a dose of 10µg / kg.b.wt. to immature rabbits for 30 days caused a significant ($p < 0.001$) increase in serum oestrogen level. Serum oestrogen level was 47.2 ± 0.6 pg/ml compared to 15.5 ± 0.3 pg/ml of the control group. Oral administration of. Oral administration of damsissa ethanol extract in a dose of 400mg/kg.b.wt. to immature rabbits for the same period produced significant ($p < 0.05$) increase in serum oestrogen. The increase caused by damsissa extract in serum oestrogen level was 30.3 ± 0.2 pg/ml compared to 15.5 ± 0.3 pg/ml of the control group (Table 4).Concerning serum progesterone level, there was an insignificant decrease in serum progesterone level in the group given the tested extract compared to the control group given olive oil.

DISCUSSION

The present study revealed that damsissa contained carbohydrates, flavonoids, tannins, triterpens, alkaloids and resins. These findings are in accordance with those of Evans [22] who reported that *Ambrosia maritima L*. (Damsissa) contains important sesquiterpene lactones and flavonoids which showed molluscicidal and cytotoxic activities and with El-Kamali and El-Amir [23] who reported that the ethanol extract of *Ambrosia maritima* contains alkaloids, flavonoids, volatile oil, 20% moisture and 20% total ash (2.5% oil Sulfated ash and 10% Acid insoluble ash).

Our study revealed that oral administration of damsissa ethanol extract to immature female mice 50 and 100mg/100gm.b.wt. significantly increased the uterine weight as compared with the control group (Olive oil). However, no literatures were found concerning the effect of damsissa extract on the uterus.

Damsissa ethanol extract when given in a dose of 400 mg/kg.b.wt. to immature rabbits for 30 days significantly increased both uterine and ovarian weights. The increased of the ovarian and uterine weights by the oral administration of the tested plant extract (Damsissa) in immature rabbits could be explained by Britt *et al.* [24] who reported that the phytoestrogens significantly increased uterine and ovarian weights of aromatase knockout mice (ArKO) which are oestrogen free.

In the present work, oral dosage of damsissa (400mg/kg.b.wt.) ethanol extracts (Which have an oestrogenic activity in mice in the present study) to immature female rabbits for 30 days enhanced ovarian activity as this extract significantly increased the average recovery rate of follicular oocytes. In addition, this plant extract increased the number of large follicles and multiple oocytes follicles (MOFS). These findings were previously reported in rats by Iguchi and Takasugi [25] and Iguchi *et al.* [26] who concluded that that exposure to oestrogenic compounds exerts increased occurrence of multiple oocytes follicles in ovary of immature mice. Moreover, Jefferson *et al.*[8] and Melissa *et al.* [27] also reported that phytoestrogen (Genistein) causes an increase in MOFS and disturb endocrine system in neonatal mice. Other investigators reported that phytoestrogens have oestrogenic [28] and anti-estrogenic properties [29] or properties independent of their oestrogenicity [30] such as suppression of follicular development (Specially in early stages), inhibition of oestradiol action, disruption of normal endocrine function, increase of androgen levels, decrease of oestrogen levels and induction of multioocytes follicles in the mature mouse ovary. Neonatal oestrogen treatment inhibited cyst breakdown suggesting multiple oocytes follicles. In addition, Krege *et al*. [31] and Julie *et al.* [32] suggested that the primary mechanism by which oestrogen elicits its action is through nuclear hormone receptors, á and â oestrogen receptors

Our results pointed to reduction in the quality of immature follicular oocytes (Grades I excellent and II good) in female rabbits given orally the studied plant extract. The reduction in the quality of oocytes may be due to the affection of cumulus-oocyte complexes (COC) to nutrient imbalance and abnormalities in the surrounding environment as reported by Thompson [33], Yamashita *et al.* [34] and Van Cauwenberge and Alexandre [35].

In vitro maturation of ovarian oocytes collected from the rabbits after oral administration of damsissa extract for 30 days showed suppression to a lesser extent of *in vitro* maturation rate and decrease of fertilization rate *in vitro*. These effects depended on the degree of cumulus cells expansion only not on the presence of 1st polar body

(Cytoplasmic and nuclear development). This means that plant extracts exerted a proliferative effect on granulose cells rather than on the cytoplasm or nucleus of oocytes. Some investigators concluded that the dose of phytoestrogenic extracts play crucial effects which may induce oestrogenic [36] or antiestrogenic properties [29] or properties independent of their estrogenicity [30]. Moreover, Tamaya [37] suggested that phytoestrogens could either mimic or antagonize the action of endogenous oestrogen. Unfer *et al.* [38] also reported that phytoestrogens increased follicle-stimulating hormone, luteinizing hormone and 17ß-estradiol plasma concentrations which may be essential for promoting of oocytes maturation *in vitro*. The present results are in consistent with other investigators who reported deleterious effects of phytoestrogens on the oocyte maturation. The most pronounced suppression of oocyte maturation by genistein and coumestrol is due to the presence of a direct relationship between the potency of the oestrogenic activity of particular phytoestrogens and the inhibition of oocyte maturation [39]. The authors added that phytoestrogens may disturb the conception and fertilization rates in cows by prolonging oocyte maturation. Sharma and Bhinda [40] found that *Trigonella foenum-graecum* seeds extract; that has also phytoestrogen activity as damssisa,exerts antiestrogenic and antifertility activity in female rats. On the other hand, oestrogen may exert its inhibitory effects on gonadotrophin action at more than one site and can modulate either the maturation-inducing action of progesterone directly at the oocyte level or the suppression of progesterone production by the follicle cell. Hence, oestrogen may play an important role as an endogenous regulator of oocyte meiotic maturation [41].

Oral administration of damsissa ethanol extract in doses of 400mg/kg.b.wt. to immature rabbits for 30 days significantly increased serum oestrogen level, while did not affect serum progesterone level. No available literature could be obtained concerning effect of damsissa extract on serum oestrogen and progesterone.

Histopathological examination of the ovaries and uteri after 30 days oral administration of damsissa ethanol extract to immature rabbits showed degenerated corpora lutea and interstitial fibrosis in the examined ovary while the uterus showed congestion of stromal blood capillaries. In this respect, no available literature could be obtained concerning effect of damsissa extract on the ovarian and uterine tissue in experimental animals given these extracts.

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