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EFFECTS OF ORAL ADMINISTRATION OF *Lepidium sativum, Moringa oleifera OILS AND* AQUEOUS EXTRACT OF *Vitex agnnus castus* ON REPRODUCTIVE PERFORMANCE AND BLOOD BIOCHEMICAL OF DOE RABBITS

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ABTRACT: *This study was aimed to evaluate the effects of dietary supplementation with Lepidium sativum (LS), Moringa oleifera Oils (Mo) and aqueous extract Of Vitex agnnus castys (VACLex) on reproductive hormones, antioxidant, biochemical blood and reproductive performance in female rabbits. Sixty Baladi red does 7- month age, with average body weight of 3250±78.2 g, were used for the present study. Rabbits were randomly divided into six equal treatment groups. The control group (1st group) was fed a basal diet without addition. The 2nd, 3rd, 4th, 5th and 6th treatments were orally supplemented 3 ml of LS, MO, VACL ex /doe/day and 3 ml of different sources of oil/doe/day combination with 3ml VACLex /doe/day, respectively for eleven consecutive day's prior insemination. Blood samples were collected through the marginal ear vein from each female rabbit prior insemination.*

Results are summarized as follows:

1- Concentration of E217β, P_4, F.T, LH and FSH hormones were increased for all treatments compared to control. However, the doe rabbits consumed MO recorded highest $E_2$17β and P_4 concentrations.

2- No statistical differences between groups supplied with VACLex with or without LS or MO on $E_2$17β, P_4, FT, LH and FSH.

3- Concentration of Total antioxidant capacity (TAC) enzyme for all treated groups supplemented with oils and /or aqueous extract was increased, whereas malonylaldehyde (MAD) enzyme concentration represented a significantly opposite trend.

4- All treated groups supplemented with oils and /or aqueous extract decreased serum triglyceride (TG),

TC and low density lipoprotein) LDL) and increased high density lipoprotein (HDL) level.

5- Concentration of TP, ALP and GLO were increased in all treated groups while, concentrations of AST, ALT and GLU were decreased.

6- Showed that all groups of doe rabbits received different oil either separated or combined with VACLex represented significant increase of sexual receptivity, conception rate, gestation length, litter size and body weight at birth in treated groups.

***Conclusively,** these results showed that inclusion of LS, MO and VACLex in doe rabbits increases reproductive hormones level and improved antioxidant status biochemical blood and reproductive performance (receptivity, conception rate and litter size).*

Key words: *Lepidium sativum, Moringa oleifera* oils ,aqueous extract, *Vitex agnnus castus* , reproductive performance, blood biochemical, doe rabbits.

INTRODUCTION

Medicinal reproductive plants are extensively used to relieve sexual dysfunction or as fertility enhancing agent, through their nutritional content known to improve sexual performance and fertility (Sumalatha *et al.,* 2010). The biological activities of the medicinal plants have been accredited to chemical constituents that induce or suppress particular physiological action in the body system (Faraz *et al.,* 2003). Medicinal plants are known as a prolific source of secondary metabolites which have important function both *in vivo* and *in vitro* during the ovarian folliculogenesis and steroidogenesis in many animal species. With the development of the technology, there is an increase implication of those substances in assisted reproductive technology (*Gildas et al., 2017*). Also, some secondary metabolites can labor antioxidants as a rule through their ability to scavenge reactive oxygen species (ROS) or can regulate ovarian hormonal production. Some plants comprise of biological actives substances which have been used to treat reproductive dysfunction (Jha *et al.,* 2010*).*

Lepidium sativum (LS) seeds contain 22.7% of oil from a hexane extraction. The oil constituents revealed oleic (30.6%), linolenic (n-3, 29.3%), gondoic (11.1%), palmitic (9.4%) and linoleic acids (n-6, 7.6%), (Moser *et al.,* 2009). Moreover, essential amino acid profile for LS includes histidine, threonine, arginine, valine, methionine, phenyl alanine, isoleucine, leucine and lysine

(Gokavi *et al.,* 2004). Also, contained of a large amount of the antioxidants, α-tocopherols (Moser *et al.,* 2009) and include a high concentration of minerals.

Lepidium sativum (LS) has been illustrated to have use for plays a pivotal role in medicinal, for instance, increase breast milk (Pullaiah, 2006), increase sexual stamina, sexual receptivity (Jabeen *et al., 2017*) and recorded to possess antifertility (Falana *et al.,* 2014), also role on antiovulatory (Satyavati, 1984).

Moringa oleifera (MO) seeds contain about 42% of a brilliant yellow, high-oleic acid crude oil having a pleasant. The oil consists of 82% unsaturated fatty acids, 70% of which is oleic acid (Rahman *et al.,* 2009). Thus high amount of unsaturated fatty acids, high oleic acid oils are known to be healthy alternatives to hydrogenated vegetable oils (Farooq *et al.* ,2006). Oleic acid was the major unsaturated fatty acid; palmitic, stearic, arachidic and behenic acids were the saturated fatty acids while palmitoleic and linolenic acids were present in small amounts (Babatunde *et al.,* 2014).

The presence phenolic compounds in Moringa seed oil is an added value to its nutritional and health potential. Furthermore, the occurrence of flavonoids in the Moringa oil which are also phenolic compounds, similarly improves the health potential of the oil. This is in agreement with previous findings which suggested that flavonoids carry out antioxidant action through scavenging (Middleton *et al.,* 2000).

Fertility in animals treated with *Vitex agnnus* extract this might be attributed to Vitex increases fertility by helping regulates hormonal and menstrual balance. Vitex is a key ingredient in pregnant in animals (Milewicz *et al.,* 1993) and improved the numbers of embryos for extract due to Vitex stimulates and stabilize the reproductive hormones involved in ovulation and assists in restoring overall hormonal balance, cycle balance, and menstrual regularity, and increase fertility by acting on the hypothalamus and pituitary gland, also turn secrete hormones or send signals to other organs of the body to excitation the production of reproductive hormones (Bergmann *et al.,* 2000). Characteristic constituents of the *vitex agnus-castus* leaf include essential oils, glycosides, flavonoids and also labdanditerpenoids, rolundifuran, vitexilactone, which have high binding affinity to dopamine receptors (Hoberg *et al.,* 1999).

Therefore, the objective of this study is to investigate the potential effect of *lepidium sativum, moringa oleifera* oils and aqueous extract of *Vitex agnnus* leaves with or without mixture between them on reproductive hormones,

performance; antioxidant status and biochemical blood constitutes of female Baladi Red rabbits.

MATERIALS AND METHODS

The present work was carried out at a private rabbit farm at Qaluobia Governorate, temperature ranged from 19 to 27°C while, humidity was 43 to 54% and light period 16 hr light: 8 hr dark.

Preparation of plant extract:

The ripened ***vitex agnus castus*** leaves were harvested and were cleaned. They were dried in sunlight and were powdered. The stock solution was made by taking 100 mg of vitex agnus castus powder /10 ml boiled distilled and mixed for 20 minutes. The mixture was filtered through a Whatman No.1 filter paper and concentrated solution using water vapor. The final concentration of the aqueous stock is each 1ml containing 100 mg of *vitex agnus castus* leaves extract (VACLex). The aqueous extract was stored in a freezer for further experiments (Harborne, 1984 and Manyfe, 2016). Assessment of effective compounds by HPLC according to Dogan *et al.* (2011).

Experimental design:

Sixty Baladi Red does 7- month age, with average body weight of 3250±78.2 g, were used for the present study. Rabbits were randomly divided into six equal treatment groups and orally administered with 3 ml of different sources of oil/doe/day and water extract of *vitex agnus castus* for eleven consecutive day's prior insemination as follows:

Group 1: Basal diet + 3 ml sterilized water was given orally and served as control group (C)

Group 2: Basal diet + orally 3 ml of *Lepidium sativum* oil (LS)

Group 3: Basal diet + orally 3 ml of *Moringa oleifera* oil (MO)

Group 4: Basal diet + orally 3ml VACLex / doe/day (VACLex)

Group5: Basal diet+ orally 3 ml of *Lepidium sativum* oil + orally 3 ml VACLex / doe /day (LS+ VACLex)

Group6: Basal diet+ orally 3 ml of *Moringa oleifera* oil+ orally 3 ml VACLex / doe /day (MO+ VACLex).

Housing and management:

The rabbits were housed in a naturally ventilated building and kept in individual wire galvanized cages (60×55×40 cm). Batteries were accommodated

with feeders for pelleted rations and automatic drinkers. Animals were kept under similar management, hygienic conditions, healthy and clinically free of external and internal parasites.

Diet nutrient profiles:

Rabbits were allowed to a standard pelleted diet (Table 1) according to NRC (1977). Feed and water were available *ad libitum.*

Table 1: The composition and chemical analysis of the basal experimental diet

Ingredients	**%**	***Calculated analysis***			
Yellow corn	6.22	Crude protein, %			18.8
Soybean meal, 44%	22.33	Crude fiber, %			13.0
Wheat bran	2345	Ether extract, %			3.0
Barley	15.00	Digestible energy (kcal/kg diet)			2680
Alfalfa hay	30.12	n-6 PUFAs%			0.3
Ground limestone	1.00	n-3 PUFAs%			1.03
Dicalcium Phosphate	1.20	***Determined analysis (g/kg)***			
Common salt	0.50	DM	897.1	CF	138.5
Vit. + min. premix*	0.30	OM	801.4	EE	26.2
Total	**100**	CP	172.4	NFE	575.0
				Ash	87.9

Each 3 kg of premix contains: Vit. A: 12,000,000 IU; Vit. D3: 3,000,000 IU; Vit. E: 10.0 mg; Vit. K3: 3.0 mg; Vit. B1: 200 mg: Vit. B2: 5.0 mg Vit. B6: 3.0 mg: Vit. B12: 15.0 mg; Biotin: 50.0 mg; Folic acid: 1.0 mg; Nicotinic acid: 35.0 mg: Pantothenic acid: 10.0 mg; Mn: 80 g; Cu: 8.8 g; Zn: 70 g; Fe: 35 g; I: 1 g; Co: 0.15g and Se: 0.3g.

DM: Dry matter, OM: Organic matter, CP: Crude protein, CF: Crude fiber, EE: Ether extract, NFE: Nitrogen-free extract,

Analysis of plants oil and* aqueous extract of *vitex agnus castus:

Data of Table 2 showed analysis the major important component of *Moringa oleifera* and *Lepidium sativum* oils and aqueous extract of *vitex agnus castus.*

Artificial insemination:

Does were inseminated using a heterospermic pool diluted 3 times (1Semen: 3 extender). A pool of semen was collected from bucks (untreated) of proven fertility for artificial insemination (AI), which performed by depositing 0.5 ml of fresh diluting semen deeply in the upper of vagina by sterile catheter. Semen was diluted with extender stored at room temperature (20° C) and used within 4 hr of collection (semen ejaculates were individually evaluated microscopically and the ejaculates which showed active progressive motility percentages (≥ 70%) were pooled and extended to Tris-buffer extender. The final concentration rate was 80 -

Table 2: Some chemical properties of *Lepidium sativum (LS), Moringa oleifera oil* (MO) and aqueous extract of *Vitex agnus castus* leaf (AVCLex)

Qualitative compounds	**Concentration**		
	LSO	***MO***	**AVCLex**
Isovitexin			
Casticin			852(µg/g,23.
Campesterol		15.1g /100g	
Stigmasterol		19.21g/100g	
β–Sitosterol		46.7 g/100g	
Campesterol		15.8 g/100g	
Total phenolic	g 36.41 mg GAE/100		
Tocopherols	2160(ug/g oil):		
linolenic acid	34.8%		
linoleic acid	11.4%		
oleic acid	21.9%		
α Tocopherols	957 ug/g		
γ Tocopherols	668 ug/g		
ǥ Tocopherols	535 ug/g		

$100\times^{10}$ motile sperm/ml. The insemination was immediately followed by the administration of 0.8 µg of buserelin IM (0.2 ml Receptal; Hoechst, Frankfurt, Germany) to induce ovulation (Lopez and Alvariño, 2000).

Moringa oleifera oil analysis according to Mahmud *et al.* (2017), Aqueous extract of *vitex agnus castus* analysis according to Dogan *et al*. (201), *Lepidium sativum* oil analysis according to Ghada *et al*.(2014).

Reproductive performance:

Sexual receptivity:

Receptivity can be computed by dividing the number of dorsiflexion of back in compliance to mounting (Does was tested in the presence of a vasectomies buck as described by the International Rabbit Reproduction Group (IRRG 2005) with total number of mounts and multiplying this ratio with 100 (Avitsur and Yirmiya,1999).

Conception rate:

Conception was tested at 14-day post mating through palpation. Conception rate was calculated as a ratio of the number of does conceived to the total number of does inseminated multiplied by 100.

Litter size and weight at birth:

Litter size and weight at birth were measured by direct counting of newborn immediately after kindling. It included number of stillbirth (Paci *et al*., 2012).

Blood biochemical constituents:

On completion of the experimental period, blood samples (5ml) were withdrawn at morning from marginal ear veins for each treatment group before access feed and water by using sterile disposal needles.

Samples collected into test tubes without heparin to collect serum for detected hormones. Blood samples were centrifuged at 3000 r.p.m for 15 min to obtain clear serum samples and stored at -20 ºC until analysis the following hormones.

***Determination hormones*:**

Serum levels of estrogen, progesterone and testosterone hormones were measured by using enzyme-linked immunosorbent assay (ELISA) kits (Diagnostics Test Canada, Inc., Ontario, Canada).

The sensitivity of hormone detection per assay tube was 10 pg/ml for estrogen and 0.1 ng/ml for progesterone according to Odell and Parlow (1981).

Biochemical analysis:

Collected serum samples were subjected to biochemical analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities (Reitman and Frankel, 1957), total protein (Sonnenwirth and Jarett, 1980), albumin (Doumas, 1971), globulin was calculated; triglycerides were assayed using the method of Fasati and Prencipe (1982).Total cholesterol was measured using the method of Stein (1986).

All biochemical parameters were analyzed by commercially available kit methods. GNW-Model: SM-721 Spectrophotometers, Absorbance Microplate Reader and other laboratory equipment aids were used for biochemical analysis. Moreover, each parameter was done according to the instructions of its kit.

Measurement of serum antioxidant:

Biochemical analyses of serum TAC and MAD were determined using commercially available kits methods using spectrophotometers, (GNW-Model: SM-721) according to Ippoushi *et al.,* (2005).

Statistical analysis:

All data were subjected to analysis of variance as described in SAS program (SAS, 2002). The significant means differences among groups were separated by Duncan's multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical analysis of oils plant and aqueous extract of vitex agnus castus:

Data of Table 2 showed the major important component of *Moringa oleifera* and *Lepidium sativum* oils and aqueous extract of *vitex agnus castus*.

Effect of treatment on reproductive hormones concentration:

Table 3 shows the effect of different sources of medical plants oils (*Lepidium sativum and Moringa oleifera*) with or without aqueous extract *Vitex agnus castus* administration for doe rabbits on $E_2 17\beta$, P_4 and F.T hormonal concentrations. The obtained data revealed that $E_2 17\beta$, P_4, F.T, LH and FSH hormones concentrations for all groups were higher ($P \leq 0.05$) compared to those for control group. However, the doe rabbits consumed MO recorded the highest $E_2 17\beta$ and P_4 concentrations compared with the other treatments and control group. Results show that there were no statistical differences between the groups supplied with aqueous extract VACLex with or without *LS* or *MO* on $E_2 17\beta$, P_4, FT, LH and FSH. While, there were a diminishing on the ratio between P4 and $E_2 17\beta$ hormones on the treated groups compared the control group. On the other hand, control group exhibited significantly the lowest concentration of $E_2 17_{\beta}$, P_4, LH and FSH.

Essential reproductive hormones (Estradaiol17β, P4 and FT.) were playing an important role with the preparation of the reproductive genital tracts for ova fertilization and the onset of secretion of LH (*Ball and Peters, 2004*). Also, progesterone is one of the majority prominent fertility hormones safe for carrying out pregnancy to period (Funston, 2004).

Current results indicated that LS oil inclusion significantly increased ($P \leq 0.05$) $E_2 17\beta$, P_4 and its ratio, which leads to increase pulsative surge of LH. These results are in agreement with those reported by Imade *et al.* (2018) who illustrated that rabbit consumed LS seeds shown increasing in plasma LH levels which were attributed to the phytoestrogens constituent in the seed. Interestingly, *Lepidium sativum* oil contains several types of polyunsaturated fatty acids (PUFA), (46.8%) and monounsaturated fatty acids (MUFA), (37.6%), (Ghada *et*

Table 3: Effect of oral administration of *lepidium sativum(LS), moringa oleifera* (MO)oils and aqueous extract of *Vitex agnus leaf* (AVCLex) on serum blood reproductive hormones of doe rabbits

Parameters	Treatment groups					
					AVCLex	
	Control	*LS*	*MO*	AVCLex	*LS*	*MO*
$E_2$17β(pg/ml)	16.41^{c} ±.68	33.45^{b} ±0.98	59.25^{a} ±1.87	35.83^{b} ±0.94	36.3^{b} ±0.91	35.1^{b} ±0.54
P_4 (ng/ml)	3.95^{c} ±0.11	5.90^{b} ±0.28	9.23^{a} ± 0.18	6.33^{b} ±0.13	6.4^{b} ±0.14	6.23^{b} ±0.15
LH(ng/ml)	46.21^{b} ±1.78	55.19^{a} ±2.67	58.37^{a} ± 0.65	61.24^{a} ±2.38	60.23^{a} ±3.40	59.96^{a} ±2.43
FSH(ng/ml)	55.23^{c} ±1.78	94.31^{a} ±3.67	89.51^{a} ±3.67	86.51^{a} ±2.67	80.34^{ab} ±3.41	81.32^{ab} ±3.22
FT (ng/dl)	0.82^{a} ±0.04	0.74^{b} ±0.03	0.65^{b} ± 0.45	0.68^{b} ±0.05	0.69^{b} ±0.0.06	0.75^{b} ±0.04
Ratio P_4/ $E_2$17β	0.241^{a}	0.176^{b}	0.156^{c}	0.176^{b}	0.176^{b}	0.177^{b}

[a,b.c.] Values with different superscripts in the same row, differ significantly (P≤0.05).E2 E217β = Estrogen ,P4= Progesterone ,LH= luteinizing hormone , FSH=Follicle stimulating hormone , FT= Free Testosterone

al., 2014). Furthermore, the unsaturated fatty acids worked as precursors of prostaglandins (PGs) synthesis and steroidogenesis (Stocco *et al*., 2005). Moreover, Mattos *et al.,* (2004) showed that feeding a diet rich in linoleic and linolenic acids could contribute to increasing secretion of PGF2α, thus causing a more complete regression of the CL (Howard *et al.,* 1990). According to Smits (2010) the follicular phase begins after luteolysis (lysis of corpus luteil) and ends at ovulation Hence, Gonadotropins (GnRH) released from hypothalamus, FSH and LH, released from the anterior pituitary developing follicles (Adams, 1999). Otherwise, secretion of LH may be ascribed to *Lepidium sativum* phytosterol constituent through temporary or permanent alteration of the feedback loop mechanism in the hypothalamus, pituitary and the reproductive gonads by simulation the effects of endogenous estrogen and trigger their specific receptors, thereby resulting in increased LH secretion. This theory agreement with the resulted of Al-Yawer *et al.,* (2006) who demonstrated that effects of LS on reproduction at the hormonal level. Interestingly, Oluwatosin *et al.* (2018) recorded that LS seeds supplementation stimulates gonadotropins secretion in

rabbits through the activation of estrogen receptors producing agonistic effects and regulate resulting increase secretion LH and FSH.

Respecting of *Moringa oleifera*, Otitoju *et al.* (2019) documented that show of *Moringa Oleifera* product led to a significant improving in estrogen level in female and maintains a healthy production of blood cells. Inversely, Nwamarah *et al.* (2015) showed that extract had its reproduction change and led to the impaired fertility in some of these rats and this effect was attributed to the intake of *Moringa oleifera.*

In the same context**,** *Vitex agnus castus, which* has antioxidant effects due to its content high levels of phytoestrogen as flavonoids, diterpenes, and volatile oil (Rani and Sharma, 2013 and Akram *et al.,* 2016), that causes significantly increased follicle-stimulating hormone, and luteinizing hormone levels. Also, Van Die *et al.* (2013) documented that aqueous extract or ethanolic extracts of Vitex have same antioxidant components, therefore it used as a treatment for premenstrual syndrome, abnormal menstrual cycles, amenorrhea, lactation poverty and decrease fertility. As well as, Gamal *et al.* (2015) recorded significant increase in the hormone concentration of LH, FSH, Estrogen and progesterone in blood serum for doe rabbits treated with Vitex agnus.

Current results indicated that *Vitex agnus castus* agreement with result obtained by Abd El Aziz *et al.* (2015) who referred that Vitex Agnus Castus showed significant increase in progesterone and estrogen. Also, Sugantha (2017) referred that use *Vitex agnus castus* led to ameliorating efficacy of the hormonal changes of polycystic ovary syndrome (POS) and improve the possibility of pregnancy.

Regarding of effects of testosterones hormone free testosterone is the testosterone molecules in blood and non conjunction to any other active molecule. High testosterone is more commonly a snag than low testosterone. It is causing many problems as polycystic ovarian syndrome, congenital adrenal hyperplasia and ovarian cancers and therefore infertility (Rachel, 2019).

The results obtained from our study showed that testosterone levels were demeinching in all treatments compared to the control group.

Concerning for progesterone to estrogen ratio, current result indicated that decreased ratio between P_4/E_2 these indicate to raise estrogen concentration. The increase concentration of E_2 objective to maintain optimal concentration of P_4 through the implantation period as well as in the luteal phase of female cycle. These hormones regulate generate cytokines, growth factors, home box

transcription factors and cyclooxygenase-derived prostaglandins through autocrine and paracrine pathways (Blazer *et al.*, 2004). Estrogen/p_4 ratio is thus an assumed marker for endometrial receptivity which up regulates adhesion zygote on the endometrial pinopods and equivalent ligands on the blastocyst for improve and successful implantation in uterus (Abuelghar *et al.*, 2013).

Effect of treatment on antioxidant activity:

Date of the antioxidant activity (Table 4) revealed that the concentration of TAC enzyme significantly ($P \leq 0.05$) increased for all treated groups supplemented with oils and /or aqueous extract compared to control group, whereas MAD enzyme concentration represented a significantly ($P \leq 0.05$) opposite trend.

The medicinal property of a specific plants can be determined by the presence of various natural compounds with bioactive potentials materials and the equiponderant proportion of these components give them therapeutic distinguishing. Also, the plant parts or extracts having major antioxidant property wherewithal greater therapeutic sectors advantageous property of a medicinal plant often depends on their property to scavenge damaging free radicals or their antioxidant potentiality (Greenwell, 2015). Results are in agreement with our study, Eman *et al.* (2019**)** who reported that *L. sativum* methanol extract effective on increasing antioxidants and ameliorating lipid profile. Also**,** Afsana *et al.* (2017) referred that seeds of *L. sativum* are good source of selenium (Se). Otherwise, Shankar and Akhilender (2015) mentioned that fed rats *Lepidium sativum* oil seed (is a rich source of α-linolenic acid, 33.6 %, the oil has a fairly balanced saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids ratio and tocopherol) led to increased catalase activity and glutathione S-transferase activities in liver significantly higher.

In same context, Moringa seed oil recorded lose on TAC and opposite trend of MAD. This finding agreed with the result reported by Bin *et al.* (2019) and Amal *et al.* (2019) who mentioned that increase antioxidant factor led to lower in serum malondialdehyde and more importantly. Also, Ojo and Adetoyi (2017) observed that the total antioxidant capacity value of rabbits increased consistently with increased *Moringa oleifera* leaf extract concentration and reduce lipid peroxidation and enhance oxidative status of rabbits. On the other hand , Sreelatha and Padma (2009 *)* suggests that the extracts of mature and tender leaves *Moringa oleifera* have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage.

Table 4: Effect of oral administration of *lepidium sativum (LS), moringa oleifera* (MO) oils and aqueous extract of *Vitex agnus leaf (*AVCLex*)* on serum blood antioxidant activity of doe rabbits

Parameters	Treatment groups					
					AVCLex	
	Control	*LS*	*MO*	AVCLex	*LS*	*MO*
TAC(nmol/ml)	1.14^{c} ±0.084	2.05^{a} ±0.092	1.84^{ab} ±0.43	1.65^{b} ±0.44	1.67^{b} ±0.44	1.75^{ab} ±0.61
MAD(nmol/ml)	7.85^{a} ± 0.53	4.67^{c} ±0.57	5.39^{b} ± 0.50	5.22^{b} ±0.78	4.83^{c} ±0.44	5.43^{b} ±0.54

[a,b.c.] Values with different superscripts in the same row, differ significantly (P≤0.05). TAC=Total antioxidant capacity, MAD =Malonyelaldehyed

Current results indicated that *V. agnus-castus* play important role on antioxidant capacity, it is contain many active materials as flavonoids, castein, orientin and isovitexin which possess the antioxidant and radical scavenging properties (Sarikurkcuv *et al* ., 2009). Also, Loch *et al*., (2000) mentioned that the methanolic extract of *V. agnus-castus* leaves exhibited major antioxidant activity in different antioxidant type, including ferric-chelating, scavenging activity of hydrogen peroxide and cupric-reducing antioxidant capacity. Interestingly**,** Özlem *et al.* (2013) illustrated that crude extracts of *V. agnus-castus* seeds have potent antioxidant, cytotoxic and apoptotic activity and reinstatement of activities of some antioxidant enzymes and reduction in the mitochondrial hydrogen peroxide production in animals therapeutic treated with the extract.

Effect of treatment on total cholesterol and triglyceride:

The data in Table (5) show the levels of serum TG and TC revealed that the control group showed a significant increase in serum concentration but significantly (P≤0.05) decreased for all treated groups supplemented with oils and /or aqueous extract compared to control group, whereas LDL concentration represented obverse trend as significantly (P≤0.05) decreased, otherwise, HDL represented increasing level for all treatment.

Triglycerides (TG) and cholesterol (TC) in the blood cause to damage vascular endothelial cells, leading to heart disease. Increasing level fat produces an increase in TG levels due to lipoprotein lipase TG hydrolysis, so that the accumulation in the liver becomes more evident (Feoli *et al.,* 2003). Data are agreement with Diwakar *et al.* (2008) who illustrated that rats consumed *Lepidium sativum oil* showed lowered serum triglycerides and cholesterol and

Table 5: Effect of oral administration of *lepidium sativum (LS), moringa oleifera* (MO) oils and aqueous extract of *Vitex agnus leaf (*AVCLex*)* on serum blood lipids profile including total cholesterol and triglycerides of doe rabbits

Parameters	Treatment groups					
	Control	***LS***	***MO***	**AVCLex**	**AVCLex**	
					LS	***MO***
TG (mg/dl)	^{a}04.801 ±6.24	5.25^{b}9 ±1.08	90.25^{b} ±1.16	7.23^{b}9 ±2.83	91.75^{b} ±2.02	.88^{b}98 ±1.72
TC (mg/dl)	89.37^{a} ±2.69	79.75^{b} ±2.09	73.50^{c} ±2.21	79.50^{b} ±2.57	85.03^{b} ±2.58	79.50^{b} ±0.87
LDL-c (mg/dl)	43.35^{a} ±1.06	28.55^{b} ±0.58	26.35^{c} ±1.29	25.97^{c} ±1.31	28.98^{b} ±1.23	27.85^{c} ±1.26
HDL-c (mg/dl)	35.42^{ab} ±0.67	50.15^{ab} ±1.24	46.17^{ab} ±1.09	52.62^{a} ±1.40	55.95^{a} ±1.08	49.77^{ab} ±1.30

$^{a,b.c.}$ Values with different superscripts in the same row, differ significantly (P ≤0.05).

TG=triglyceride, TC=Total cholesterol, HDL= High-density lipoprotein cholesterol, LDL=, low-density lipoprotein cholesterol

increased the rate of HDL, it have revealed presence of glycoside, alkaloids, phenolic compound, flavonoids and amino acids as glutamine, cysteine, and glycine. The phenolic (tannin) and flavonoids may have antioxidant activity where it is considered glutamate, cysteine, glycine are intermediates for synthesis of the antioxidant glutathione (Hamer and Steptoe, 2006). On the other hand, Amal *et al.* (2019) reported that *Lepidium sativum* exert major affect and modify on lipid profile. Interestingly, Amawi and Aljamal (2012) reported that significant decreasing value of cholesterol, triglycerides, LDL and increase in HDL. In the same context, Ahmed *et al.* (2015) discover that extract Vitex *Agnus Castus* extracts, led to significantly decreased (P≤0.05) TC, TG and LDL-c and increase HDL-c in mice. Same result obtained by Berrani *et al.* (2018) show that oral administration of methanolic extract of Vitex Agnus have an improuve on lipide profile in rate. Concerning, *Moringa oleifera* leaves, *Shahidi et al. (1992)* reported that its a rich source of β-carotene, protein, vitamin C, calcium, potassium and act as a good source of natural antioxidant due to the presence of ascorbic acid, flavonoids, phenolics and carotenoids.

Results revealed that *Moringa Oleifera* extract had improved the serum lipid profile by decreasing serum TC, TG, LDL and increasing serum HDL in rabbits agreed with Samar *et al.* (2016) who showed a significant diminishing in

Table 6: Effect of oral administration of lepidium sativum (LS), moringa oleifera (MO) oils and aqueous extract of Vitex agnus leaf (AVCLex) on liver function and serum proteins of doe rabbits.

Parameters	Treatment groups					
	Control	*LS*	*MO*	AVCLex	AVCLex	
					LS	*MO*
AST (U/L)	42.20[a] ±2.45	32.52[b] ±3.21	31.55[b] ±2.89	33.21[b] ±1.45	31.25[b] ±1.66	33.25[b] ±1.34
ALT (U/L)	28.32[a] ±1.87	23.2[2b] ±1.73	22.25[b] ±.99	23.25[b] ±1.65	23.51[b] ±0.89	23.85[b] ±1.12
TP(g/dL)	6.13[b] ±0.32b	7.11[a] ±0.41	7.17[a] ±0.38	7.14[ab] ±0.44	7.09[a] ±0.65	7.01[a] ±0.59
Alb (g/dl)	3.40[b] ±0.31	3.92[a] ±0.29	3.84[a] ±0.21	3.72[a] ±0.31	3.95[a] ±0.23	3.87[a] ±0.27
Glo (g/dl)	2.73[b] ±0.25	3.29[a] ±0.38	3.33[a] ±0.31	3.42[a] ±0.45	3.14[a] ±0.31	3.68[a] ±0.42
(mg/dL) Glu	158.81[a] ±3.21	144.23[b] ±4.12	139.44[b] ±3.38	138.25[b] ±3.34	148.81[b] ±3.41	145.72[b] ±3.89

[a,,b.c.] Values with different superscripts in the same row, differ significantly (P≤0.05).
ALT=Alanine minotransferase, AST=Aspartate aminotransferase, TG =Triglycerides, TP=,Total protein, Alb=Albumen, Glo= Globulin,Glu= Glucose

total TC and LDL. Same result obtained by Idemudia *et al.* (2013) who found that HDL level increased in rats fed on *Moringa Oleifera*.

Effect of treatment on blood biochemical:

Table 6 showed the effect of different treatment oils with or without aqueous extract *vitex agnus castus* administration for does on AST, ALT, TP, ALB, GLO and GLU biochemical blood level. Data revealed that TP, ALP and GLO concentrations for all groups were higher (P≤0.01) compared to those for control group. However the doe rabbits of AST, ALT and GLU recorded a decreasing concentration for the all groups treated with oils and extract compared to those for control group. Results show that there were no statistical differences between oils with or without extract Mix oils groups with respect to GLU concentration. On the other hand, control group exhibited significantly the highest concentration of AST, ALT and GLU compared supplemented groups.

Results of in vivo expriment indicated that administration of différent oils with or without extract has a profound influence on the liver function, metabolism

of charpohydrate and protein in doe rabbits. It could be suggested that the use of plant oils and extract could improve liver function and métabolisme.

As regards the effect *Lepidium sativum* oil**,** our result agree with Ghada, *et al.* (2014) who refereed that albino rate fed with supplemented diet *Lepidium sativum* significant decrease in AST and ALT. Shivangi *et al.* (2017) mentioned that the treatment with *Lipidium sativum* was establish to be most effective in expression of improvement in biochemical parameters as ALT, AST and significant higher TP, ALP and GLO.

Concerning, *Moringa oleifera,* our date are in agreement with Fathy *et al.* (2017) who showing significantly increase on total protein (TP), albumin (ALP) in albino rats. Also, Voemesse *et al.* (2018) referred that supplemented fed with *Moringa oleifera* leaf were significantly increased total protein and albumin levels. Result obtained by supplemented aqueous extract vitex agnus castus agreed with Berrani *et al.* (2018) who showed that oral administration of methanolic extract of decreased blood glucose after 2 h. Contrary, Franciele *et al.* (2015) recommended that glucose levels were not significantly modified by supplemented with *Vitex Agnus Castus.*

Effect of treatment on reproductive performance:

Date of Table 7 showed that all groups of rabbit does received different oils either separated or combined with aqueous extract of Vitex Agnus Castus represented significant ($P \leq 0.01$) increase of sexual receptivity, conception rate, gestation length, litter size and body weight at birth in treated groups compared with control. However, rabbits of Moringan Oleifera group significantly ($P \leq 0.01$) recorded lowest litter size at birth and L.W at birth. Also, results showed that there were no statistical differences between all treat groups with respect to previous mentioned parameters while, control group recorded lowest values for all studied parameters.

The present results are agreement with Imade *et al.* (2018) who mentioned that consumed high level of Lepidium Sativum seed significantly increased conception rate and decrease gestation length ,litter size at birth and increase ovulation fertilization also implantation because all the rabbits were receptive at the time of mating. Also, Jabeen *et al.* (2017) reported that to increase sexual stamina (receptivity) and sexual receptivity. Bin *et al.* (2019) recorded that *Moringa oleifera* leaf improved litter size, litter birth weight and litter survival. Contrariwise, Musa *et al.* (2014) reported that supplemented rabbit diet with Moringa oleifera led to significantly lower litter size but higher weight at birth

Table 7: Effect of oral administration of *lepidium sativum(LS), moringa oleifera* (MO) oils and aqueous extract of *Vitex agnus leaf (*AVCLex*)* on reproductive performance of doe rabbits

Parameters	Treatment groups					
					AVCLex	
	Control	*LSO*	*MO*	AVCLex	*LSO*	*MO*
Sexual receptivity (%)	65^{c} ±1.80	84^{a} ±1.30	85^{a} ±1.5	81^{a} ±2.9	82^{a} ±3.9	80^{a} ±2.1
Conception rate (%)	72^{b} ±1.71	85^{a} ±1.13	73^{b} ±1.82	83^{a} ±1.21	85^{a} ±1.43	81^{a} ±1.12
Gestation length (day)	.213 ±0.60	30.1 ±0.50	31.3 ±0.60	31.2 ±0.75	31.2 ±0.40	31.3 ±0.80
Litter size at birth (n)	6.2^{b} ±0.40	8.4^{a} ±0.70	$^{b}16.$ ±0.37	8.9^{a} ±0.79	$^{a}19.2$ ±1.20	$^{a}59.1$ ±1.30
LW at birth (g)	42.4^{b} ±1.5	42.1^{b} ±0.9	48.2^{a} ±0.8	47.5^{a} ±0.95	49.20^{a} ±0.90	47.12^{a} ±1.3

$^{a,,b.c.}$ Values with different superscripts in the same row, differ significantly (P≤0.05).
LWB =Litter weight at birth

and same gestation period are similar for control group. On the other hand, Munif *et al.* (2016) observed that the Vitex agnus castus had a major role in fertility in doe albino rabbit by inhibition of the prolactin hormone as the main cause of injuring the women by infertility.

Conclusively, these results showed that inclusion of LS, MO and VACLex in doe rabbits increases reproductive hormones level and improved antioxidant status biochemical blood and reproductive performance (receptivity, conception rate and litter size).

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تأثير التجريع الفموى لإناث الأرانب بزيت حب الرشاد والمورينجا ومستخلص كف مريم على الاداء التناسلى والكيمياء الحيوية للدم

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** قسم بحوث الثروة الحيوانية، معهد بحوث زراعة المناطق الجافة - مدينة البحث العلمي والتطبيقات التكنولوجية ، الإسكندرية ، مصر.

إستخدم فى هذه الدراسة عدد 60 أنثى أرنب من سلالة بلدى أحمر عمر 7 شهور بمتوسط وزن 3250 ± 78.2 جم بشكل عشوائي إلى ستة مجموعات متساوية. تم تغذية الأرانب في كل المجموعات على عليقة الأساسية دون إضافات. تم تجريع الأرانب فى المجموعات الثانية والثالثة والرابعة والخامسة والسادسة ب 3 مل من كل من زيت حب الرشاد أو زيت المورينجا أو مستخلص كف مريم أو 3 مل من مستخلص كف مريم مع 3 مل من كل من زيت حب الرشاد أو زيت المورينجا، على التوالى لمدة 11 يوم متصلة قبل التلقيح.

ويمكن تلخيص النتائج على النحو التالي:

1- زاد تركيز كل من هرمون الإستراديول والبروجستيرون و LH, FSH فى كل المجاميع المعامله مقارنة بالكنترول بينما سجلت المجموعة المعاملة بزيت المورينجا أعلى معدل لكل من هرمون الإستراديول والبروجستيرون.

2- ليس هناك أى تأثير إحصائى على مستوى هرمونات الإستراديول والبروجستيرون و LH, FSH لكف مريم مع أو بدون زيت حب الرشاد أو زيت المورينجا.
3- حدث تحسن فى حالة مضادات الأكسدة فى كل المجاميع المعاملة مقارنة بالكنترول. الكنترول.
4- زادت مستويات البروتين الكلى والألبيومين والجلوبيولين بينما إنخفضت إنزيمات الكبد لكل المجام
5- إنخفض كل من الدهون الثلاثية والكوليسترول الكلى ومكوناته للمجموعات المعاملة مقارنة مع مجموعة يع المعاملة مقارنة بمجموعة المقارنة.
6- لوحظت زيادة كل من قابلية الإناث للتلقيح ومعدل الحمل وطول فترة الحمل وحجم ووزن الخلفة عند الميلاد فى كل المجاميع المعاملة مقارنة بمجموعة المقارنة.

التوصية: استخدام زيت حب الرشاد أو زيت المورينجا ومستخلص كف مريم بالتجريع لإناث الأرانب أدى لزيادة مستوى الهرمونات الجنسية وحالة الأكسدة، كما أدى لتحسن صفات الدم والأداء التناسلى.