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Assessment the Estrogenic Efficacy of L-carnitine on Female Rats treated with Sodium Fluoride

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ABSTRACT
This investigation has been carried out for determine the estrogenic activity of L-carnitine, and role in female reproductive system in sodium fluoride treated rats. Mature forty female rats were isolated randomly to 4 groups while were taken care every day as follow of 30 days; group T1 control were administered tap water ;group T2 administered sodium fluoride in tap water 100 ppm; group T3were intubated daily L-carnitine 100mg/kg B.W; group T4 were intubated daily L-carnitine 100mg/kg B.W and administered sodium fluoride in tap water 100 ppm. Blood samples were obtained at end of experiments for estimation of serum estrogen, progesterone ,FSH, LH concentrations. Samples from both organs were also obtained for histo-physiological study (estimation ovarian & uterine weights, diameter the graafian follicles and percentage intact and atretic graafian follicles also thickness of the uterine wall layers). A significant decrease in serum estrogen,progesterone, FSH, LH concentration, also, the ovarian and uteri weights in group T2, as well as, statistical analysis and examination of histological sections revealed a significant decrease in the diameter and percentage of intact graafian follicles , also thickness of the uterine wall layers and increase in percentage of atretic graafian follicles. Whereas, The results pointed the beneficial effects of LC to improvement the previous parameters, against NaF (T4), through a significant increase in hormonal profile concentration and the ovarian and uteri weights same results were also recorded in the thickness of all uterine layers (endometrium, myometrium and stroma) parallel with alleviating the toxicity induced by NaF.

Keywords: L-carnitine, fluoride, FSH, LH, Estrogen, rats

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INTRODUCTION
Fluoride is striking for its little size; enormous quantities of fluoride molecules fit around atoms of another component. Beside, electronegativity permits forming of numerous simple & complex fluorides [1]. Sodium fluoride(NaF) is a sturdy, hard,anion associated a accumulative toxic agent an inorganic anionic compound, on dissolving give separated ions Na and F[2]. Additionally largest contributor drinking water, in foods are rich sources of fluoride[3]. NaF is employed in numerous pesticide formulation, included fungicides and pesticide in addition to wood preservative [4]. Lately, in modernistic preventive dental medicine fluoride is used as a new agent for management of tooth decay[5]. Fluoride be affect on the reproductive system of animals[6].. In female rats with exposure to NaF in drink water, important decreases within the variety of viable fetuses and will increase within the resorption rate be observe [6]. Additionally, fluoride reveal to induce free radical toxicity in mice ovary[7]. Excessive exposition into environmental pollutants and chemicals is a main principle to reproductive health problems[8]. Fluoride is a common naturalistic pollutant with certain lethal impacts, the association between long-range fluoride exposition and fertility weakness has been catch to concern [9].

Levo-carnitine could be a biologically active water soluble antioxidant molecule [10] that comes from dietary supply (75%) and internally synthesis from essential amino acids (Lysine and Methionine)(25%)[11]. Carnitine belong to one of the spesific categories from nutrients referred to as 'quasi-vitamins' or 'conditionally-essential 'nutrients it's existing in whole mammals, centralized on the inner mitochondrial membrane [12] and facilitates transmit of LCFAs via mitochondrial membrane for utilization in energy generation (β-Oxidation).

L-carnitine(LC) features a potent antioxidant property and is employed for preventing and treatment of oxidative stress[13]. It protects the cells from apoptosis and ameliorates a lot of the mechanisms of physiological antioxidants [14]. Antioxidant properties of L-carnitine seems clearly in additional than one mechanism, for instance decreasing the production of ROS [15] ,maintenance of antioxidant enzymes (SOD, CAT and glutathione peroxidase)[16]L-carnitine includes a potent scavenger capability for free radicals. LC intake is wide considered one in all the foremost effective ways in which to rise perseverance, burning fat and abbreviate post-exercise recuperation [17]. In peripheral tissues, its assistance at β-oxidation via transmit medium and LCFAs to mitochondria[18]. In addition to the preserve cell wall constancy via it participitation at acylation of membrane phospholipids and amphipathic activity [19]. L-carnitine additionally prevents deoxyribonucleic acid damage evoked by the harmful actions of free radicals[20]. additionally, works as a stabilizer of depressed acetyl CoA/CoA concentration ratio also as an acetyl store for provide energy in metabolic pathways. In accordance with this, the study was centered around to investigating the estrogenic efficacy of L-carnitine on the performance reproductive tract in female rats treated with sodium fluoride.

MATERIAL AND METHODS
Chemicals
L-carnitine were purchased from medical pharmacy (Nutrition's Pride, U.S.A.), sodium fluoride purchased from Gonane office for medical devices-Iraq, BDH Co. (England).

Animals
Adult female Swiss albino rats the age of these ranged between (8-10 weeks) in age and the weight about (175-250gm) were used in this investigation were housed in cages with good ventilation and lightening in the animale place of the College of Basic Education-University of Wasit . Water and standard rodent chow were freely available.

Experimental design

Female forty Swiss albino rats division randomly at 4 identical groups and were taken care every day as follows for 30 days; group T1 control were administered faucet water ;groupT2 administered sodium fluoride in faucet water 100 ppm; groupT3 were intubated daily L-carnitine 100mg/kg B.W[21] ; groupT4 were intubated daily L-carnitine 100mg/kg B.W and administered sodium fluoride in faucet water 100 ppm.

At end of experiment blood sample were collected via cardiac puncture technique then centrifuged at 3000 rpm for 15 minutes , and sera were isolated and frozen at -18C° till analysis of serum estrogen, progesterone, (FSH), LH concentrations by using immunoenzymometric assay kits (monobind Inc , USA). Rats were sacrificed through withdrawal of blood from heart. Immediately, after sacrification ,laparotomy were perform, and the all reproductive system be rapidly isolated then soaked in a petri dish full with normal saline, kept at 37 ºC. Both ovaries were rapidly dissected out using fine surgical scissors, cleaned from surrounding non-ovarian tissue, and dried at filter paper, weighed in a sensitive electronic balance and recorded. Uterus cleaned from surrounded tissue, dried by a filter paper and weight were recorded by a sensitive electronic balance [22]. Then fixed with ovaries in Bouins solution for 24 hours then the organs were transferred to ethanol alcohol 50%for 1-2 hours and after that transferred to ethanolic alcohol 70% kept there until used for a histological preparation [23]. Data were analyzed statistically using analysis of variance (ANOVA) test on the basis of one way analysis of variance at a significant level of p<0.05. Specific group differences were determined using least significant differences (LSD) [24].

RESULT

The table 1 showed administration of L-carnitine alone group T3 or concurrently with NaF in tap water group T4 caused significant (P<0.05) elevation in concentration of reproductive hormones (progesterone, estrogen, FSH, LH) comparative to the values at group T2. On the other hand, the result illustrated highly significant (P<0.05) depression in reproductive hormones after NaF exposure group T2 in comparative to the value of other treated groups. Beside, statistical analysis recorded non-significant variations (P>0.05) in the level of reproductive hormones in-group T4 as compared to the control.

The data show a marked significant (P<0.05) increment in ovarian weight and uterine weight in L-carnitine treated groups T3 compared with others experimental groups, while group T4 showed non-significant differences (P>0.05) comparing to control group. Conversely, the result recorded highly a significant (P<0.05) decrement in there parameters of rats exposed to sodium fluoride at concentration 100 ppm as compared to other groups table (2) .

Table(3)and figure(1)shows that the group of L-carnitine recorded significant increments (P<0.05) in the mean percentage of the intact graafian follicles and decrease in atretic follicles as compared to the other treated groups. Moreover, it is worthy to mention that significant (P<0.05) decreases at NaF treated group(fig.2) exhibited in the mean percentage of intact graafian follicles and increase in atretic follicles as compared to the other treated groups. In addition to that significant (P<0.05) increase in the mean diameter (µm)of graafian follicles of L-carnitine treated group as compared to the control and other treated groups was recorded, while NaF treated group appear a significant (P<0.05) decrease in the mean diameter (µm)of the graafian follicles comparative to the control and group T4 figure (5).

Significant increments (P<0.05) in the uterine layers in T3 group as compared to other groups recorded as shown in table(4)and figure(3).On the other hand T2 group(figure 4) recorded a significant decrease (P<0.05)in comparing to the other treated groups. However, histological section demonstrated a significant increase in the uterine layers (figure 6) was found in L-carnitine plus NaF treated group as compared to T2 group.

Table 1: Effect of L-carnitine on serum reproductive hormones concentration (progesterone, estrogen, FSH and LH) in normal, sodium fluoride treated female rats.

Group	Progesterone	Estrogen	FSH	LH
T1	2.17±0.01 B	27.17±0.01 B	12.01±0.09 B	4.15±0.02 B
T2	1.20±0.02 C	22.20±0.02 C	6.10 ±0.29 C	1.27± 0.06 C
T3	3.22±0.02 A	30.22±0.02 A	15.22±0.02 A	5.28±0.02 A
T4	2.20±0.03 B	27.15±0.04 B	11.90±0.16 B	4.25±0.05 B
LSD	0.07	0.08	0.13	0.13

Values expressed as mean ±SE; n: 10; Different capital letter represent a significant difference between groups (p <0.05) vs. Control; T1: Control; T2: sodium fluoride (100ppm); T3: L-carnitine (100mg/kg B.W); T4: sodium fluoride (100ppm)+ L-carnitine (100mg/kg B.W).

Table 2: Effect of L-carnitine on ovarian and uterine weight in normal, sodium fluoride treated female rats.

Group	Ovarian weight	Uterine weight
T1	5.11±0.05 B	32.31±0.14 B
T2	3.27±0.06 C	23.27±0.06 C
T3	6.08±0.22 A	40.28±0.02 A
T3	4.93±0.18 B	31.93±0.28 B
LSD	0.45	0.49

Table 3: Effect L-carnitine on diameter of the graafian follicles and percentage of intact and atretic graafian follicles in normal, sodium fluoride treated female rats.

Group	% of intact graafian follicles	% of atretic graafian follicles	Diameter graafian follicles (µm)
T1	59.11±0.26 B	40.17±0.02 C	228.10±0.28 B
T2	12.27±0.06 D	79.87±0.25 A	160.68±0.19 D
T3	68.68±0.24 A	15.68±0.24 D	320.18±0.03 A
T3	55.73±0.29 C	42.73±0.26 B	226.66±0.25 C
LSD	0.70	0.66	0.64

Table 4: Effect of L-carnitine on thickness of the uterine wall layers (µm) in normal, sodium fluoride treated female rats

	Thickness of Uterine layers(µ)		
Group	Epithelial	Stroma	Myometrium
T1	20.30±0.10 B	292.16±0.04 C	66.17±0.02 C
T2	13.88±0.28 D	242.24±3.72 D	57.87±0.27 D
T3	25.60±0.22 A	396.26±2.43 A	80.88±0.38 A
T3	18.46±0.19 C	323.85±2.06 B	75.13±0.22 B
LSD	0.64	7.3503	0.78

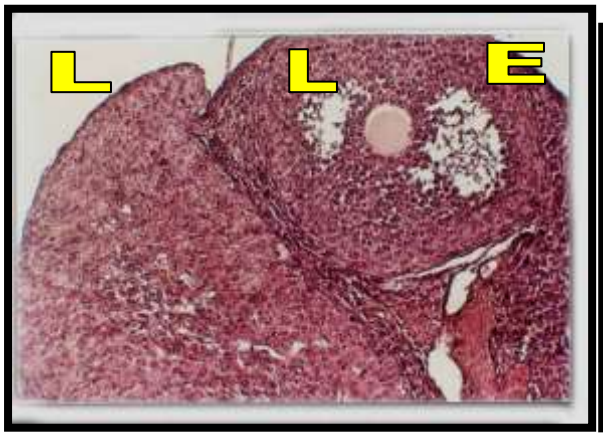

Figure 1 a: section of ovary group T3
E: intact of graafian follicle
L: Active corpus luteum
(H. and E. stain X50)

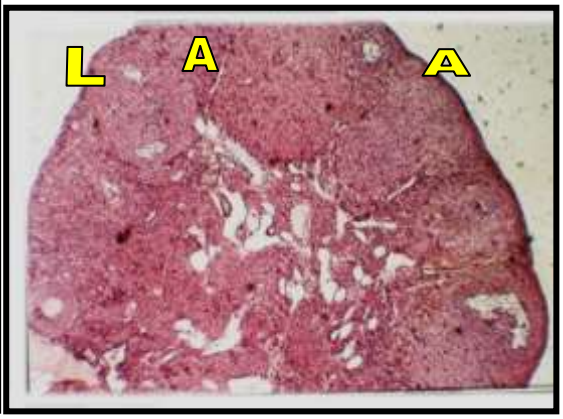

Figure 1 b: section of ovary group T3
A: atretic graafian follicle
L: Active corpus luteum
(H. and E. stain X20)

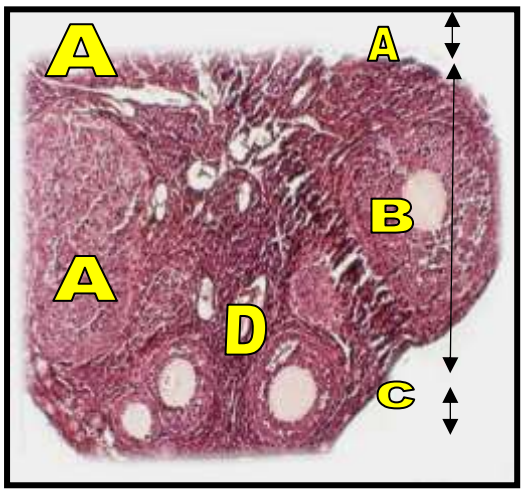

Figure 2: section of ovary group T2
B: Beginning of degenerative corpus luteum
A: atretic follicles
C: Secondary follicles
(H.& E. stain X50)

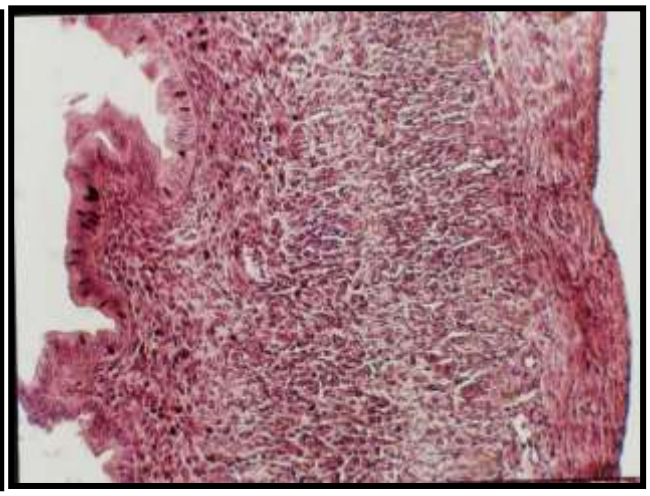

Figure 3: section of uterus group T3
A: Epithelial lining of endometrium
B: Developed stroma of endometrium
D: numerous number of uterine glands
C: - thick myometrium layer

(H. and E. stain X50)

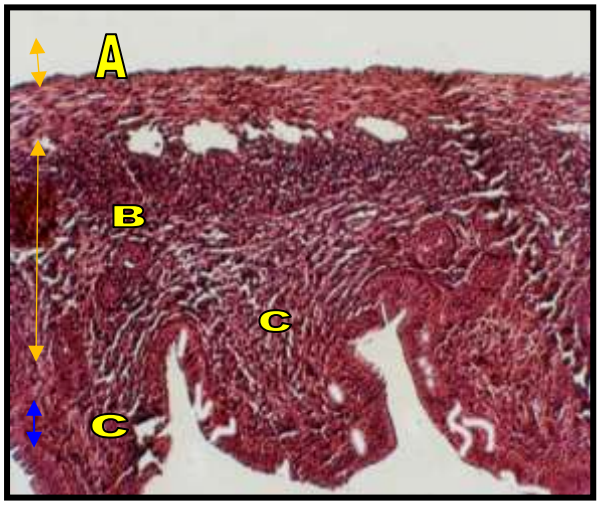

Figure 4: section of uterus group T2

A: Thin layer of myometrium
B: poorly developed stroma of endometrial layers
C: destructed and sloughed epithelial layer of endometrium
(H. and E. stain X50)

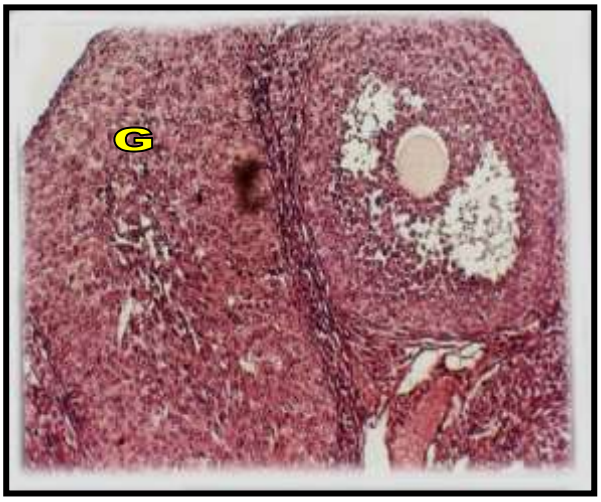

Figure 5: section of ovary group T4

G: Graafian follicle
C: Active corpus luteum
(H&E stainX50)

Figure 6: section of uterus group T4 -Epithelial lining layer(a)Stroma of endometrium(b)-Normal myometerium (c)

DISCUSSION

Exposure to NaF is associated with impairment function of reproductive system organs in both human and experimental animals especially the Gonads[25]. The dominant mechanism of action of the toxic effects of NaF occurs at the level of the hypothalamic/pituitary axis interrupting gonadotropic hormones production (FSH and LH)leading to disturbances of ovarian function. The sharp blockade of steroidogenesis by NaF is may be because uncoupling of the LH receptor and adenylate cyclase, and impaired cholesterol utilization by mitochondrial cholesterol side chain cleavage enzyme(P450sec.) through suppression of protein synthesis [26]. While the L-carnitine treated group recorded a significant increament in serum hormones level due to the capability in creating a suitable statues for hormone synthesis and release through hypothalamic/ pituitary/ gonadal axis, or may be by direct effect on the ovarian tissues inducing the steroid hormone synthesis and release[27], also L-carnitine enters the oocyte via OCTN2 and by its direct action on oocyte quality[28], this raise energy production by β-oxidation, remove excessive palmitate from ER to reducing stress, scavenges free radicals for reduce oxidative stress and suppress caspases to avoide apoptosis, beside it ameliorates hormonal balance, reduce the release of cytokines and apoptosis [29]. Carnitine concurrently with NaF was registered a significant increment and decrement in steroid hormones as compared to NaF and L-carnitine treated groups respectively, the elevation of steroid hormones may because to role of L-carnitine to decrease the negative effects of toxicity of NaF also may be due to increase of NaF effects or toxicity, thus NaF have accumulative effect so that the impact of L-carnitine was decrease compared with the high toxicity of NaF.

It is worthy to mention that significant increases in ovarian and uterine weights in the L-carnitine group .This might be ascribed to the antioxidant role of the L-Carnitine, probably due to hypothalamus or pituitary gland stimulation through receptors other than those of endogenous estrogen. The estrogenic potency was determined by induction a significant increment in uterus weights[30]. While,NaF group register a significant decrease in the ovarian and uterine weights because the oxidation lead to apoptosis, atresia of various uterine cells, and a decrease in the follicles and corpora lutea percentages the major participants in the ovarian weight, also indicated that initiation of peroxidation process in ovarian and uterine tissues lead to FRs formation accompanied by an antioxidant capability depression, apoptosis and atrophy that was reflected by weight loss[9]. Histological sections indicated that L-carnitine antioxidant activities in scavenging free radicals, which is accompanied with a detectable morphological and functional changes in various cellular components current study is in accordance with other studies[31].Those results indicated that L-carnitine could act on hypothalamic/pituitary axis inducing gonadotropic hormones secretion, therefore graafian follicles development stimulation and maturation since follicular growth through folliculogenesis in adult undoubtedly achieved by FSH and LH[30]. While increase in the thicknesses uterine layers are compatible and reflect ovarian changes that involved significant increases in the intact graafian follicles and finally increase in the synthesis and secretion of ovarian hormones inducing uterine growth and development[32]. While, NaF exerting oxidative damage in ovaries and uterus, in addition to the direct cytotoxic effects in uterine tissue, leading to the development retardation accompanied with Beginning of degenerative corpus luteum, destructed and sloughed epithelial layer of endometrium (fig 2,4), might be ascribed to the high levels of free radicals generation accompanied with antioxidants level depletion. Hence, most of these changes are associated with the

idiopathic gonadal failure and probably due to induced gonadotoxicity[9,33].Moreover NaF caused cytotoxicity, apoptosis and generates reactive oxygen species, which oxidize and damage a variety of cellular constituents and induced mitochondrial dysfunction [34].In Conclusion LC has potent role in improvement on female reproductive organs functions against NaF.

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