AMELIORATIVE ROLE OF SILYMARIN EXTRACTED FROM *SILYBUM MARIANUM* SEEDS ON NICKEL CHLORIDE INDUCE CHANGES IN TESTICULAR FUNCTIONS IN ADULT MALE RABBITS.

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ABSTRACT

This study aimed to investigate the Ameliorative effect of ethanolic extracted of silymarin from *Silybum marianum* seeds and to compare it's with the commercial silymarin extract(legalon) against oral dosing of Nickel chloride effects on sperms concentration, motility ,viability, sperm abnormality and male fertility and gonadal hormones .Twenty adult male rabbits aged 5 to 6 month and weighted 1.250-1.500 kg divided into four equal groups,1st group served as control group received 1 ml of normal saline (NaCl 0.9%),2nd group received (1mg/100g B.W)$NiCl_2$ orally, 3rd group received same dose of $NiCl_2$ plus (0.1mg/100g B.W) silymarin extract, the fourth group received same dose of $NiCl_2$ plus (0.1mg/100g B.W) legalon for 35 days, Results showed negative effects of $NiCl_2$ which caused significant($p \leq 0.05$) decrease in sperm concentration ,viability, motility and fertility while sperm abnormality was significantly increase, also $NiCl_2$ caused significant ($p \leq 0.05$) decrease in serum progesterone, estradiol and testosterone. while silymarin extract and legalon adverse the negative effects of $NiCl_2$ and causing ameliorative effects on all the studied parameters .

INTRODUCTION

Milk thistle (*Silybum marianum,* family: Compositae is an annual plant native to the Mediterranean area, North African regions which have now spread to other warm and dry regions (**1**).as well as it grows in north part of Iraq and north of Bagdad (**2**). The most important medicinal application of milk thistle is its use as a protective and as supportive treatment of chronic inflammatory liver disorders such as cirrhosis, hepatitis, and fatty infiltration due to alcohol **(3,4)).** and toxic effect of chemicals like lead **(5).**Exposure of animals and humans to different metal

components through contaminated drinking water can result in a wide range of adverse clinical conditions**(6).** Rabbit males have relatively low fertility rate as compared to other mammals. Since rabbits have low fertility so they may be at greater risk from reproductive toxicants **(7). (8)**stated the big problem of low fertility in rabbits.

Nickel (Ni) is a heavy metal present in parts of the environment. It is the fifth most widespread element on Earth.(**9**). **(10)** stated that Nickel reduced growth rate, reduced reproductive rates, and alterations of serum lipids and glucose have been observed in animal studies Administration of nickel chloride to young male mice. (**11)** found nickel observed effects on sperm motility and count there also increase in abnormal sperm count at the same dose levels.

MATERIAL AND METHODS

Twenty sexually mature male rabbits bought from the local market of Basrah city of 5 to 6 month age weight 1.250-1.500 kg caged individually in metallic cages and randomly divided into 4 group treated for 35 day. 1st Control group : five male rabbits were served as control group and received 1 ml normal saline(NaCl 0.9%) orally. 2nd group: given 1mg /100gram Body weight(B.W) $NiCl_2$ orally. 3rd group : given 1mg /100gram B.W $NiCl_2$ followed by 0.1mg/100gram B.W ethanolic extract of silymarin. 4th group: given 1mg /100gram B.W $NiCl_2$ followed by 0.1mg/100gram B.W Leganol**(**commercial silymarin seed extract , named legalon forte from MADUS GmbH, Colgen,Germany **).**After the end 35 days of treatment 5 ml of blood sample were collected by heart puncture and serum isolated for hormonal estimation. Male rabbits were anesthetized and surgically castrated ,the isolated epididmyis were put in petry dish contain 5 ml of Normal saline(0.9% $NaCl_2$) and cut into small pieces to make suspension, the suspension filtrated by clean gaus into test tube. 2ml aspirated and semen deposed artificially in the vagina of untreated healthy female by small catheter , the rest semen collected from the epididmyis used for seminal analysis and artificial insemination for untreated healthy females rabbits in which ovulation induced by intramuscular injection of 50 iu HCG(human chorionic gonadotropin) (HCG 5000 Rate company - France) .

Seminal analysis:

Neubauer Hemocytometer chamber was used for Sperm count in semen collected from epididmyis according (**12)**.Sperm viability was measured by eosin–nigrosin(14) **.**

Percentage of abnormal spermatozoa is measured by using same slids used for viability measurement 200 spermatozoa were counted using 40x objective.

Male fertility is determined by measuring the percentage of fertility using the following formula:

$$\text{Fertility percentage} = \frac{\text{Number of delivering females}}{\text{Number of mated female}} \times 100$$

Hormonal estimation :

Determination of serum gonadal hormones were measured by ELISA (Enzymes-linked ImmunosorbentAssay by ELISA Kits(Human-Germany),Estimation of testosterone(**13**),estradiol.(14).and progesterone **(15).**

The Statistical analysis

The results of the present study were analyzed by using one way analysis of variance (ANOVA) test. The statistical analysis was performed by using the program(SPSS) and chi-square for fertility statistical analysis. The data were expressed as a means ±SE. ($P<0.05$) were considered to be significant for all data of this study.

RESULTS AND DISCUSSION

The results of the present study (table 1) showed a significant ($p\leq0.05$) decrease in sperm concentration ,motility, viability and increase sperm abnormality in male rabbits that received 1mg/100g B.W nickel chloride orally compared with control group as well as with males received silymarin extract or legalon.

Table (1) The effect of pre oral supplementation of $NiCl_2$, $NiCl_2$ plus silymarein extract and $NiCl_2$ plus Legalon on sperm concentration ,viability and abnormality(Mean ± SE).

Parameter / Group	Sperm concentration x10^6	Motility %	Viability %	Abnormality %	Fertility %
Control NaCl 0.9%	242.6 ±7.054 A	82 ±2.00 A	74 ±2.449 A	4.60 ±0.871 B	100
Group1 $NiCl_2$(1mg/100g B.W	126.2 ±5.631 B	44 ±5.099 B	45± 5.000 B	27.60± 2.039 A	0.0
Group 2 $NiCl_2$(1mg/100g)+SN ext.(0.1g/ 100gB.W)	254.6 ± 7.054 A	78 ± 2.00 A	70± 0.010 A	4.80 ± 0.860 B	80
Group 3 $NiCl_2$(1mg/100g)+ legalon.(0.1g/100gB.W)	251.6 ±7.054 A	84 ±2.449 A	59.60±3.04 AB	2.60 ± 0.509 B	90
LCD	116.400	34.00	25.00	22.80	

N=5 male, female SN =Silymarin, Capital letters denote Mean differences is significant at level ,P≤0.05 vs.

The reproductive health of animals could be affected by a number of endogenous as well as exogenous factors, such as exposure to heavy metals(**17**).Thousands of metals and chemicals have been released into the general environment and the oral exposure of these metals caused severe damage in male reproductive health.(**18).**

Nickel was found to be responsible on many sexual disorders **(19). (20)** indicated that nickel(1mg/kg) when administrated to rats and goats impaired reproductive performance and significantly decreases spermatozoa motility , density in the epididmyis, epididmyal transit time of spermatozoa, testis spermatozoa production and dietary nickel (1 mg/kg) can damage sperm.. **(21)** Indicated significantly decreased in spermatozoal motility of bovine after exposure to higher nickel concentrations ((1000 uM Ni ml^{-1})caused apoptosis in the spermatozoa head (acrosomal and postacrosomal part).

(22) Dosing of nickel chloride for 35 days to groups of young male mice reduced sperm motility and increased abnormal sperm . Degeneration of the germinal epithelium of the rats testes was observed only at the much higher concentration of 1.6 mg Ni/m3 in male rats exposed for 6 h/day for 12 days **(23).** Metals may pentrate the blood barrier and baddly spermatogenesis intigrity or hormone production leading to low sperm motility , densety and increased morphological anomalies and male infertility**(24).**In mouse nickel chlorid caused apoptosis in testes as well as affect the function of the somniferous epithelium at the site of spermatozoa production **(25).** Nickel chloride in drinking water in rats(10-100ppm) for 28 days caused shrinkage of the seminifrous tubules and decreased in the basal spermatogonia(**26**). A significant positive correlation between the percentage of tail defects in spermatozoa and blood nickel concentration .In human nickel caused sperm abnormalities**(27)** . Table (1) also showed that fertillity was highly affected by Nickel chloride dosing in (group 2), diminished pregnancy compared with control(100%) and the other groups, that indicated the ameliratave effect of silymarin extract(80%) and legalon 90%)(**26**) found that males exposed to $NiCl_2$ (for 28 or 42 days before copulation),resulted in reduced both the number of pregnancies and the number of pups born(**28**) found that Ni – induced decreases infertility and alteration of testicular steroidogenesis in male rats. (**29)**showed that $NiCl_2$, induced on day 21 of pregnancy a progressive diminution of the number of live fetuses with 25 and 50 mg /kg, B.W, S/C; this

diminution was reached it's maximal value with 100 mg/kg, in comparison with control.

(30) indicated that silymarin prevented pregnancy in female rats and caused some histological changes in the ovary and uterus, while it has biological benefits for male rats during short treatment pointed that heavy metals transported into the egg by spermatozoa may also pose a significant risk to the developing embryo via their toxicity .The present study suggested that a higher number of damaged spermatozoa may reduce sperm kinetic characteristics and probably fertilizing capacity by triggering specific morphological damages to the head and/or by inhibiting motility

Table(2)The effect of oral Dosing of $NiCl_2$, $NiCl_2$ plus silymarein extract and $NiCl_2$ plus Legalon on serum progesterone, estradiol and testosterone .(Mean ± SE)

Parameters / group	Progesterone ng/ml	Estradiol pg/ml	Testosterone ng/ml
Control Normal 1 ml saline (0.9 NaCl)	0.3920±0.00646	19.2220±.28150	0.5500±.01390
Group1 $NiCl_2$(1mg/100g B.W	0.2810±.02079	16.8160±.68404	0.3650±.01558
Group 2 NiCl(1mg/100g)+silymarein ext.(0.1g/ 100gB.W)	0.4890±.00836	19.7980±.68145	0.4660±.01579
Group 3 $NiCl_2$(1mg/100g)+leganol.(0.1g/ 100gB.W)	0.4100±.00650	21.8960±.68008	0.3760±.01454
LSD	0.0790	2.09	0.084

N=5 male, female SN =Silymarin, Capital letters denote Mean differences is significant at level ,P≤0.05 vs.

The data in Table 2 showed significant($p \leq 0.05$) decrease in serum levels of progesterone ,estradiol and testosterone after 35 days of nickel chloride dosing compared with control group, and the other groups that received nickel chloride and silymarin extract (group 2) and that which received nickel chloride and legalon. Researches focused that heavy metals such as cadmium (Cd), arsen (As), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) defined as Endocrine disrupters , Their effects may be achieved by interferences with the biosynthesis or activity of several endogenous hormones **(31)(17).** Nickel was found to be responsible on many sexual disorders(**19**) . **(32)(33)(34)** showed that $NiCl_2$ in rats treated groups exhibited significantly and noticeably lower serum concentrations of testosterone when compared with the control group. In our study both silymarin and legalon treated

group showed improvement in studied parameters in table (2). (**30)** found that male rats treated with silymarin for one month, testosterone and LH were increased significantly. **(35)** in his study on mice testicilar tissue indicated that silibinin can improve some testicular parameters as well as caused significant increase in testosterone level these studies results agree with the present results (**25)** suggested alterations of spermatogenesis directly affecting epithelium and influencing interstitial cells (increased ratio of apoptosis) producing testosterone . **(36)** pointed that toxic effects of oral exposure to nickel showing a possible impairment the development and reproductive functions. (**37**)found that after $NiCl_2$ treatment the rat interstitial (Leydig) cell culture showed dose dependent depression in both HCG- and cAMP-stimulated testosterone production so that destructions of leydig cells and sertoli caused by nickel may lead to lower serum level of testosterone and estradiol while ethanolic extract of silymarin and legalon stabilized their serum level.

CONCLUSION

Interestingly, data of the present study showed that silymarin extracted from *silybum marianum* caused an improvement in some semen quality and quantity and male gonadal hormones .In adult male rabbits treated with nickel chloride leads to increase fertility of treated males when intact females were artificially inseminated by their semen..

الدور المحسن للمستخلص الكحولي لبذور نبات شوكة مريم *Silybum marianum* ضد تأثير كلوريد النيكل الحاث للتغيرات في وظائف الخصية في ذكور الارانب

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الخلاصـــــة

تهدف هذه الدراسة لمعرفة الدور المؤثر للمستخلص الكحولي لنبات شوكة مريم مقارنة مع المستخلص التجاري للسيمارين (ليجالون) ضد تأثير التجريع الفموي لكلوريد النيكل على تركيز وحركة وعدد النطف الحية والمية والغير طبيعية والخصوبة وهرمونات التناسل في الذكور . عشرون ارنب ذكر بالغ قسمت الى اربع مجاميع تجريبية متساوية، المجموعة الاولى (مجموعة السيطرة) جرعت ب ١مل من الحلول الملحي الفسلجي ٠.٩% ،المجموعة الثانية جرعت ١ملغم من كلوريد الصوديوم /١٠٠غرام من وزن الجسم ، المجموعة الثالثة جرعت ١ملغم من كلوريد الصوديوم /١٠٠غرام من وزن الجسم يليه تجريع ٠.١ملغم/١٠٠غرام من وزن الجسم

من مستخلص السيمارين ، المجموعة الرابعة جرعت ١ملغم من كلوريد الصوديوم /١٠٠غرام من وزن الجسم يليه ١ملغم/١٠٠غرام من وزن الجسم من السليمارين التجاري (ليجالون) لفترة ٣٥ يوماً. اظهرت النتائج التأثير السلبي لكلوريد النيكل حيث سبب انخفاضاً معنوياً في تركيز النطف والحركة والنطف الحية والخصوبة بينما ظهرت زيادة معنوية في نسبة النطف الغير طبيعية، كما اظهرت النتائج انخفاضاً في مستوى بروجسترون واترادايول وهرمون التستستيرون ، بينما عكس كلاً من مستخلص السليمارين والليجالون التأثير السلبي لكلوريد النيكل.

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