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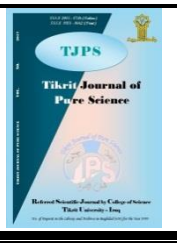

Silymarin effect as an antioxidant to improve damages induced by CCl4 on some characteristics of male rats reproductive system

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Abstract

This study was conducted to investigate if silymarin can prevent the adverse effect of oxidative stress induced by carbon tetrachloride(CCl4) on some characters of male rat reproductive system. Twenty male albino rats were divided into 4 groups, 5 for each included, control group, CCl4 group, silymarin group and silymarin plus CCl4 group. Groups with silymarin received dose (150mg/kg) and group with CCl4 received (3ml/kg) at the end of experiment. The period of the study was 35 days. CCl4 can induce male reproductive toxicity through damage testis structure, weights, reduction in epididymis, sperm count, morphology, viability and motility. Silymarin plays its antioxidant role with scavenging free radical resulted by CCl4 and showed significant increasing in body and testis weight, normal sperms counting and vital sperms in compared to CCl4 group.

Introduction

Infertility and its related problems are a major disturbing issues in people life[1]. The common cause of infertility in males is their inability to produce a sufficient number of active sperms [2,3]. Many studies results approved that oxidative stress can affect sperm production and reduce sperm count by production of free radicals and oxidation of germ cells in the testis tissue [4,5]. Studies have shown that using antioxidants can effectively treat infertility in males through reduction of damages caused by free radicals, strengthening the blood-testis barrier and protecting and repairing of sperm DNA [5,6]. The effectiveness of herbs has been validated during the past decades through modern research. These herbs generally contain antioxidants, cell membrane-stabilizing agents, or nutrients that inhibit the depletion of endogenous antioxidants such as glutathione. Silymarin is the major active compound from milk thistle extract (*Silybum marianum*).It is consists of seven flavonoglignans (silibinin, isosilibinin, silychristin, isosilychristin and silydianin) and a flavonoid (taxifolin) which is a unique type of bioflavonoid and an antioxidant that scavenges damaging free radicals. [7].
studies have been done till now on the therapeutic and biological properties of silymarin. Among its properties can point to anti-inflammatory, antioxidant, anti-cancer and hepatoprotective properties [8,9]. Silymarin plays antioxidant role with scavenging free radical as well as increasing the levels of glutathione peroxidase and superoxide dismutase (SOD) [10].
This study was performed to investigate if silymarin can prevent the adverse effect of oxidative stress induced by CCL4 on some characters of male rat reproductive system.

Materials and methods

Twenty healthy male albino rats with an average weight of 200-250 g were used in this experimental study. During the experiment, the rats were kept in the condition of 12 hours of light and 12 hours of darkness. Water and food were given to them without any restrictions during the period of the experiment which was (35) days. The animals were then divided randomly into four groups 5 for each including control, CCl4 group was given (3ml CCl4) before 24 hours of the test end, silymarin group and silymarin plus CCl4 group. Groups with silymarin received dose (150mg/kg) of silymarin.
At the end of the test and after weighing, the rats were anesthetized using chloroform and blood samples were directly taken from hearts using a 5 ml

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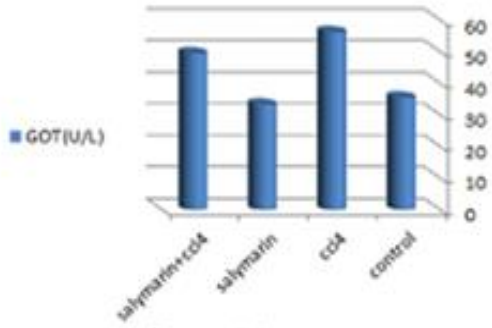
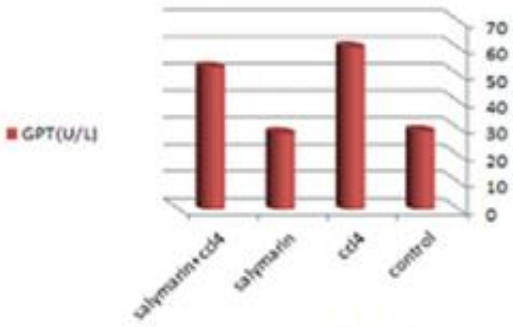
syringe. The blood serum was then collected using centrifuge (3000 rpm for 15 min) and kept at-20°C. serum were used to measure creatinine GOT and GPT levels by spectrophotometer and analysis kits. The testis were removed and tissue sections obtained. The sections were then stained with Hematoxylin and Eosin stain. counting and checking the cells were made using light microscopy. epididymis pounding in physiological solution, slides made to determine viability and mortality of sperms using eosin-nicrosin stain. Haemocytometer were used to find total sperm count using eosin stain.

Data to each group were recorded and analyzed using SPSS and one-way analysis of variance (one-way ANOVA) test. $P<0.05$ was used to show significancy. Data in the section of Results were calculated and compared as Mean±SEM.

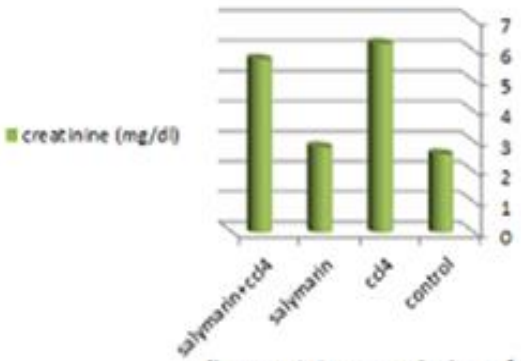
Results



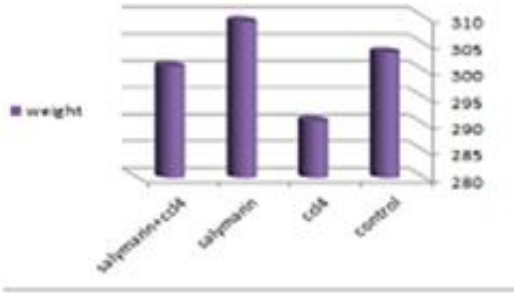
figure(1) **GOT(U/L)**



figrue(2)**GPT(U/L)**



figure(3)**creatinine (mg/dl)**



figure(4)**weight**

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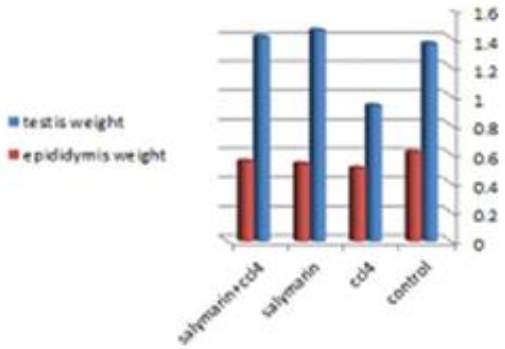
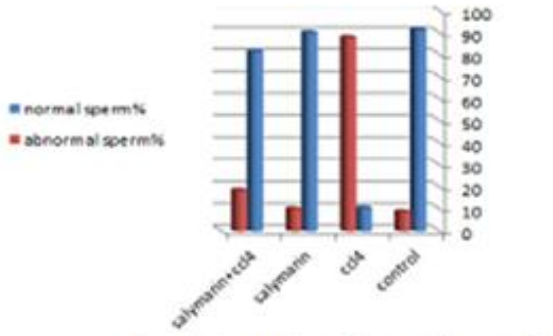
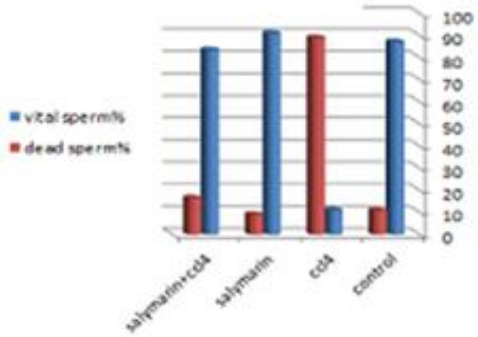


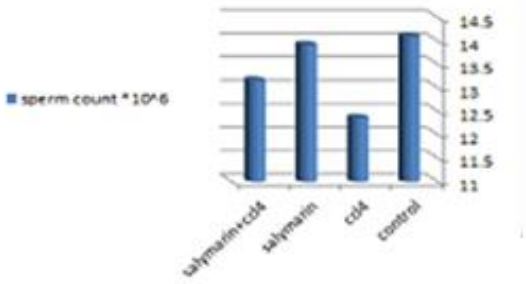
figure(5) weights of testis and epididymis)



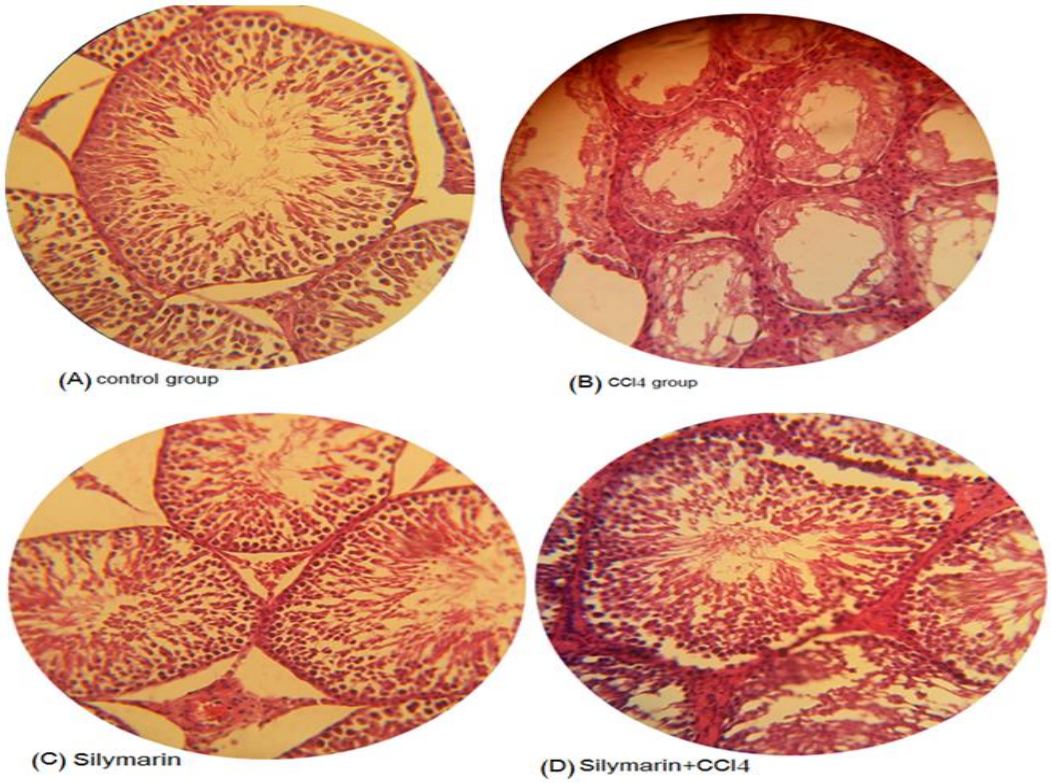
figure(6)normal and abnormal sperm %



figure(7) vital and dead sperm%



figure(8)**sperm count *10^6**



figure(9). photograph of tisticular tissue (H&E.,40x)

The average level of GOT ,GPT, Creatinine, normality of sperms , epididymis weight vitality and sperm count (figure:1,2,3,5,6,7, 8) in Group received silymarin showed no significant change at level $P \leq 0.05$ compared to the control group,While there were increasing in body and testis weight (figure:4,5).

Group received CCl4 showed a significant increasing in GOT, GPT, creatinine, sperm abnormality and mortality (figure: 1,2,3,6,7)compared to the control group, where CCl4 group showed a significant decreasing in body and testis weight, normal sperm, sperm vitality and sperm count (figure: 4,5,6,7,8) at level P≤0.05 compared to control group. CCl4+ silymarin group showed a significant decreasing in level of GOT, GPT, cratinine abnormality and mortality of sperms (figure:1,2,3,6,7) in compared to CCl4 group, while there were a significant increase in body an testis weight, normal sperm, sperms counting and vital sperms (figure:4,5,6,7,8) in compared to CCl4 group .In CCl4 group, testis seminiferous tubules (figure 9-B) are almost empty of spermatozoa. Most cells have damage to cellular components. CCl4+silymarin group (figure 9-D) Most of the cells in the stages of morphological transition , the tail of the sperm are thin and the sperm offspring are similar to the normal state but less dense than the normal state. Silymarin group
Tissue (figure9-C) is nearly similar to the control(figure 9-A).

Discussion

CCl4 can impair male reproductive function by inducing oxidative stress(11). Silymarin is a potent antioxidant and could compensate the adverse effect of CCl4 on GOT,GPT enzymes creatinine (figure 1,2,3) and some sperm characters (figure 9-B) .Viability and motility are important parameters of mature sperm which indicate its structural and functional quality to move toward an egg for successful fertilization (12). CCl4 can induce male reproductive toxicity through damage in testis structure ,decrease in testosterone level(13), testis and epididymis weights, reduction in epididymis sperm count, normal morphology, viability and motility (figure 4,5,6,7,8) .CCl4 is proposed to exert its cytotoxicity by free radicals generation and the activation of oxidative sensitive signaling pathways, which lead to a hepatotoxity in result to form intermediate radicals Trichloromethyle CCl3 which metabolize by cytochrome p450.(13). Generating free radicals cause peroxidation of hepatic membrane and raise of liver enzymes (GOT,GPT) and increasing in creatinine level(figure 1,2,3) because of inhibiting of insulin level which induce cells to use proteins as a main source for energy production in addition to the increasing of oxidation(14).

silymarine is a polyphenolic flavonoid with a potent antioxidant property which not only acts as free radical scavenger but also increases the capacity of cell antioxidant enzymes (10,12). Silymarin has long been used as a hepatic protective remedy (15) . In addition to central effects of silymarin on hypothalamus – pituitary - testis axis, increased testosterone levels in the study showed related silymarin to synthesis and metabolism of this hormone. Silymarin is counted as a potent inhibitor of aromatase enzyme Aromatase catalyzes the conversion of testosterone to estrogen. By inhibiting this enzyme, [16]. Investigation showed improving on testis tissue under effect of silymarin (figure 9-C). Studies on silybin (one of the structural isoforms of silymarin) effects on testicular tissue of laboratory white mice showed that this flavonoid is able to improve the testicular parameters, It has been shown that silymarin increased the concentration of norepinephrine, serotonin and dopamine in certain areas of the brain of laboratory white mice [17]. increasing of gonadotropin hormones from pituitary gland is related to increased release of norepinephrine by silymarin. Norepinephrine by increasing the synthesis of nitric oxide will increase releasing of GnRH from hypothalamus and LH and FSH hormones from pituitary gland [18,19] .Testosterone is a important factor of spermatogenesis process [16]; so, an increase in sperm count and density is detected due to the enhancement of the level of the mentioned hormones by silymarin. The sperm membrane is so sensitive to damages caused by oxygen free radicals [20]. Since free radicals produced in daily reactions of the body are more effective in reducing the count and motility of sperm ,one of probable mechanisms of silymarin effects on enhancement of sperm count(figure, 8) may be caused by the antioxidant properties of it. Studies results show that antioxidants in male reproductive system reduce oxidative stress in testis and increase the activity of Leydig cells and so resulting in increase of secretion of testosterone and improvement of spermatogenesis process [21,22]. The additive or synergistic effect of silymarin on the content of glutathione peroxidase has been approved in the various tissues of rats [23,24]. Glutathione peroxidase (GPX) which plays a special role in protection of sperms and ductus deferens in testis [25].

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تاثير السلمارين كمضاد للاكسدة في تحسين الضرر المستحدث بوساطة رباعي كلوريد الكربون على بعض خصائص الجهاز التكاثري الذكري للجرذان البيض

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الملخص

انجزت هذه الدراسة لمعرفة كفاءة السلمارين في منع التاثير الضار للاجهاد التاكسدي الذي يحثه رباعي كلوريد الكربون CCl4 على بعض خصائص الجهاز التكاثري لذكور الجرذان البيض. عشرون ذكراً من الجرذان البيض قسمت الى اربع مجموعات، خمس حيوانات لكل منها، تضمنت المجموعات المختبرية، مجموعة السيطرة، المجموعة المعاملة بـ(CCl4 3m/Kg)، مجموعة السلمارين (150mg/Kg) ومجموعة السلمارين مع CCl4. كانت مدة الدراسة 35 يوما. اظهر CCl4 تاثيرا سميا على الجهاز التكاثري من خلال تضرر نسيج الخصية وانخفاض وزن البربخ ،عد النطف،الشكل الطبيعي للنطف، حيوية وحركة النطف. اظهر السلمارين فعاليته المضادة للاكسدة لازالة الجذور الحرة الناتجة عن تاثير CCl4 ولوحظ ارتفاعا معنويا في وزن الجسم والخصى، النطف الطبيعية، عد النطف وحيوية النطف بالمقارنة مع المجموعة المعاملة بـCCl4.