

Protective Effect of Allium Ampeloprasum Against Toxicity Induced by CCL4 in Male White Rats

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Abstract— The protective effect of Leek (*Allium ampeloprasum*) extract against testicular toxicity induced by carbon tetrachloride CCL4 was studied. The experiment was conducted on 36 male rats distributed randomly into 6 groups of 6 animals: group (1) was kept as a normal control which received distilled water; rats of groups (2) and (3) were given only Leek extract only in a dose 200 and 400 mg/kg; and groups (4,5,6) received CCL4 at a concentration of 1 ml/L by oral administration for induction of toxicity on rats. Groups 5 and 6 received Leek extract 200 and 400 mg/kg with CCL4 respectively. Results showed that oral administration of Leek extract in rats with testicular toxicity by CCL4 increased the weight of testes and accessory genital glands, sperm motility, count and viability, serum testosterone, luteinizing hormone and follicle stimulating hormone. It could be concluded that treatment with Leek extract produced a protective effect against toxicity induced by CCL4 in the male rats.

Index Terms— Allium ampeloprasum, CCL4, Male fertility, Rat, .

1 INTRODUCTION

Infertility is one of the major health problems in life and approximately about 30% of this problem is due to male factors [1]. Several factors can interfere with spermatogenesis, reduce sperm quantity and quality and lower male fertility. Many diseases and conditions such as coronary heart diseases, diabetes mellitus, chronic liver diseases, chronic smoking, alcohol intake, prolonged exposure to industrial and insecticide contaminants, air pollutants and insufficient vitamins intake have deleterious effects on spermatogenesis and production of normal sperm [2,3]. On the other hand, some previous studies reported that the intake of antioxidants and vitamins E and C can protect sperm DNA from oxidative stress of free radicals in rats [4], and antagonized testicular toxicity caused by a pyrethroid lambda cyhalothrin insecticide [5]. *Allium* vegetable has been used as folk medicine since ancient times. Thus studies and clinical research have shown that regular consumption of a variety of examples has several beneficial health effects [6,7]. Some components of *Allium* vegetable are reported to block several stages of carcinogenesis taking into account that the underlying mechanisms of action are generally unclear [8].

Allium genus has over 500 members which differ in maturing, color and taste; however, they are similar in biochemical content.

Alliums were revered to possess anti-bacterial and anti-fungal activities because of the powerful antioxidants, sulfur and other numerous phenolic compounds [9].

Leek (*Allium ampeloprasum* var. *porrum*) belongs to the Alliaceae family, look a bit like a huge spring onion and have a mild onion flavor but have flat leaves instead of tubular and relatively little bulb development. The thick leaf bases and slightly developed bulb look like a giant green onion and are eaten as cooked vegetable [10]. Today leeks are grown as an excellent substitute for onion and for its own unique mild onion flavor in soups and other dishes. Since leeks are related to garlic and onions, they contain many of the same beneficial compounds. Leeks are a good source of allyl sulfides which have been shown to modify certain pathways associated with the growth of malignant tumors. Leeks are also rich in the saponins, kaempferol, carotenoids and chlorophyll mainly in the green tops [11]. The present study was designed to investigate the effect of alcoholic extract of *Allium ampeloprasum* on male fertility and testosterone, luteinizing hormone and follicle stimulating hormone levels in CCL4 toxicity rats.

2 MATERIALS AND METHODS

Chemicals: Carbon tetrachloride (CCL4) was obtained from the Dept. of Chemistry at Kufa university. Leek leaves were obtained from the local market.

The rats received a dose of 1ml/kg of CCL4 that was suspended in olive oil (1:1v/v) by oral administration method. Besides, leek extract was given at a dose of 200 mg/kg, 400 mg/kg by the same method.

Preparation of leek extract: leek leaves were dried at (45°C) and crushed to powder by using a blender; 100g of powdered leek leaves were added to 500ml of 80% ethanol and the mixture is placed in a soxhlet system during 24 hrs. After that, the resulting extracts were filtered using filter paper and concentrated to dryness in rotary evaporator at room temperature and the recipient was used by several dilution [12].

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2.1 Experimental Design

36 male albino rats strain (*Rattus norvegicus*) weighting 230-250g were obtained from the animal house at the Faculty of Science/Kufa University. The rats were kept under observation for one week before starting the experiment for acclimatization. They were fed on standard diet and water ad libitum. Then the animals were divided into six groups of six rats in each. The first group was fed on the basal diet and served as a normal control. The three groups were given carbon tetrachloride (CCL4) for induction of toxicity in reproductive system; CCL4 was diluted in an equal volume of olive oil as a vehicle and given by oral administration method in a dose of 1 ml/kg of body weight. The first CCL4 group was fed basal diet and kept it as toxicity group while the other toxicity groups were fed on basal diets that substitute 200,400 mg/kg of leek extract. The rest of two groups were fed on basal diets that substitute 200 and 400 mg/kg of leek extract only. At the end of experiment period (50 days), rats were anaesthetized by chloroform and blood samples were collected by heart puncture and put into serum tubes in the room temperature for several minutes and were centrifuged for 20 minutes at 3000 rpm. The sera, which were kept at -10°C till hormonal analysis, were separated. Testes, prostate glands and seminal vesicles of the sacrificed rats were taken for obtaining weight and epididymis for measurement of sperm parameters.

2.2 Semen Analysis

Epididymial contents of treated rats were obtained after cutting the tail of epididymis, squeezing it gently on clean slide and the sperm progressive motility and cell count were determined [13]. Microscopical examination of the seminal smears (stained with Eosin Nigrosin stain) were conducted to determine the percentage of sperm viability (ratio of alive/dead) and sperm cell abnormality [14]. Hormones analysis are measured by ELISA method [15].

2.3 Statistical Analysis

Data were expressed as mean±S.E., and Statistical Analysis was conducted by using computerized SPSS program version (17) with one way ANOVA [16].

3 RESULTS AND DISCUSSION

The obtained results showed that oral administration of CCL4 to normal rats were significant as it decreased the weight of body, testes, seminal vesicles and prostate glands induced marked testicular degeneration, lowered semen quality and quantity. Besides it decreased testosterone, luteinizing hormone and follicle stimulating hormone. In the present study, CCL4 - induced testicular toxicity was identical to that previously reported in rat [17]. The study here was instigated by earlier study in which the CCL4 administration caused testicular atrophy, degeneration of germinal layer, decrease in testosterone, and gonadotropins (LH,FSH) in male rats. This action of CCL4 on the testes may be ascribed a direct toxic action of CCL4 on the tissues and is likely to impair gonadal response

to LH and FSH [18].

Also, CCL4 administration for 50 days induced a decrease in weight of body and accessory sex organs. The decrease in these reproductive organs weight could be attributed to the decrease in testosterone level as a result of oxidative damage [17, 19]. while oral administration of *Allium ampeloprasum* extract in doses of 200 and 400 mg/kg to toxicity rats for 50 days significantly increased ($p < 0.05$) the weight of the body, testes and seminal vesicle but not affected the weight of prostate glands as compared to toxicity rats by CCL4.

TABLE (1)

EFFECT OF ORAL ADMINISTRATION OF *ALLIUM AMPELOPRASUM* EXTRACT FOR 50 DAYS ON THE WEIGHT OF SEXUAL ORGANS OF MALE TOXICITY RATS.

Treatment	Body weight(g)	Testes	Seminal vesicles	Prostate glands
Normal control	273±9.15	2.81±0.23	1.76±0.17	0.66±0.01
CCL4 group	218±8.21	1.67±0.14 a	1.01±0.11 a	0.23±0.02 a
200 mg/kg of leek extract	276±3.03 b*	2.25±0.13 b*	1.65±0.10 b*	0.55±0.06 *
400 mg/kg of leek extract	280±7.10 b*	2.40±0.20 b*	1.87±0.12 b*	0.63±0.03 *
200 mg/kg of leek extract + CCL4	247±7.13 b*	2.10±0.12 b*	1.51±0.16 b*	0.35±0.04 b
400 mg/kg of leek extract + CCL4	425±11.1 b*	2.15±0.18 b*	1.12±0.15 b*	0.42±0.01 b

Values are mean±S.E. Values in the same column sharing the different letters are significantly different with normal control. * significantly different with CCL4 group.

Data in Table (2) showed the oral administration of CCL4 to normal rats induced a significant ($p < 0.05$) decrease in serum testosterone, gonadotropins (LH,FSH), and oral administration of *Allium ampeloprasum* extract at 200 and 400 mg/kg to toxicity rats caused significant ($p < 0.05$) increase in testosterone, gonadotropins (LH,FSH) hormone levels as compared to toxicity rats of CCL4. Concerning *Allium ampeloprasum* extract, the obtained results showed that it is oral administration at 200 and 400 mg/kg for 50 days to male toxicity rats increased the weight of testes, seminal vesicles and prostate glands, as well as testosterone, luteinizing hormone and follicle stimulating hormone levels associated with an improvement of sperm motility and quantity, in addition to alleviation testicular degenerative changes that seen in the testis of toxicity rats. These findings agreed with those reported by Khaki et

al,2009 [20] and Morakino et al,2008 [21] who concluded that Allium ampeloprasum may be promising in enhancing healthy sperm parameters. The authors attributed the improvement of reproductive functions of male rats by Allium ampeloprasum to its antioxidant and androgenic activities. The alleviation of testicular lesions, which were seen in toxicity rats after oral administration of Allium ampeloprasum extract that reported in this study, may be explained by the previously reported of the antioxidant and androgenic effects or of the increase in testosterone, gonadotropins (LH,FSH) levels in oral administration of Allium ampeloprasum could be attributed to the direct on the central nervous system and gonadal tissues or their effects on hypothalamus-pituitary-testis axis [22, 23].

TABLE (2)

EFFECT OF ORAL ADMINISTRATION OF ALLIUM AMPELOPRASUM EXTRACT FOR 50 DAYS ON SERUM TESTOSTERONE, LUTEINIZING HORMONE AND FSH STIMULATING HORMONE OF MALE TOXICITY RATS.

Treatment	T (µg/ml)	LH (µg/ml)	FSH (µg/ml)
Normal control	3.17±0.17	1.77±0.22	20.25±1.33
CCL4 group	1.46±0.25 a	1.14±0.14 a	17.13±1.20 a
200 mg/kg of leek extract	3.19±0.27 *	1.80±0.40 *	18.45±1.50 *
400 mg/kg of leek extract	3.32±0.29 *	1.82±0.24 *	19.23±0.32 *
200 mg/kg of leek extract + CCL4	2.16±0.13 b*	1.36±0.06 b*	19.76±1.14 *
400 mg/kg of leek extract + CCL4	2.32±0.20 b*	1.51±0.18 b*	21.17±1.30 *

Values are mean±S.E. Values in the same column sharing the different letters are significantly different with normal control. * significantly different with CCL4 group.

Concerning the semen picture, it was found that CCL4 decreased sperm progressive motility and sperm count, and increased the percentage of sperm cell abnormality in toxicity rats. Also the results showed that the oral administration of Allium ampeloprasum extract (200 and 400 mg/kg) for 50 days to toxicity of rats increased the sperm progressive motility, sperm count the percentage of sperm cell abnormality as shown in the Table (3). The improvement in fertility parameters that was caused by large dose of Allium ampeloprasum extract could be attributed to its previously reported antioxidant activity studied by Moselhy and Ali, 2009 [24], Yang et al. 2006 [25]. Those scientists concluded that the natural antioxidants can protect DNA and other molecules from cell damage induced by oxidation and can improve sperm quality and increase reproductive efficiency of men. Also AL-Shalaby and Samar (2011) [18] concluded that Allium ampeloprasum ex-

tracts have antioxidant affect in CCL4 -intoxicated rats. Moreover, Jedlinska et al.(2007) [4] reported that the intake of antioxidants and vitamins A,B,C and E can increase stability of testicular blood barrier and protect sperm DNA from oxidative stress caused by active free radicals. In addition, the enhancement of fertility properties which were produced by Allium ampeloprasum extract could be explained by its direct effect on the testes causing an increase in testosterone secretion which is reported in this study.

TABLE (3)

EFFECT OF ORAL ADMINISTRATION OF ALLIUM AMPELOPRASUM EXTRACT FOR 50 DAYS ON EPIDIDIMIS SEMEN PICTURE OF MALE TOXICITY RATS.

Treatment	Motility(%)	Count(× 10 ⁶)	Viability(%)	Abnormality(%)
Normal control	90.01±1.02	93.67±0.48	89.10±0.12	3.62±0.18
CCL4 group	50.12±1.03 a	55.11±0.23 a	60.11±0.16 a	8.25±0.09 a
200 mg/kg of leek extract	88.12±2.30 b	90.66±0.42 b	85.30±0.10 b	3.33±0.17
400 mg/kg of leek extract	93.11±0.22 b	95.76±0.27 b	87.14±0.13 b	3.75±0.15
200 mg/kg of leek extract + CCL4	70.21±0.13 b*	75.22±0.34 b*	72.31±0.15 b*	5.40±0.16 b*
400 mg/kg of leek extract + CCL4	78.14±0.18 b*	84.30±0.28 b*	74.13±0.17 b*	4.24±0.22 b*

Values are mean±S.E. Values in the same row sharing the different letters are significantly different with negative control. * significantly different with CCL4 group.

4 CONCLUSION

It could be concluded that the oral administration of Allium ampeloprasum extract to toxicity male rats increased the weight of testes, seminal vesicles and prostate glands; improve semen quality and quantity and increased testosterone, luteinizing hormone, follicle stimulating hormone levels in serum. Therefore, this study recommends the intake of Allium ampeloprasum in food as it is useful for patients who suffer from sexual impotency.

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