ARTICLE

PROTECTIVE ROLE OF LETTUCE (*Lactuca sativa* L.) ON ETHANOL ALCOHOL CONSUMPTION IN MALE REPRODUCTIVE SWISS MICE

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ABSTRACT

The present study aimed to evaluate the protective effects of Lettuce (Lactuca sativa L.) against the toxicity caused by ethanol consumption in reproductive system of mice. The histopathological changes in testis and epididymis antioxidant enzymes activities of catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MAD) and glutathione peroxidase in testis-homogenized tissue were investigated. Twenty mice were categories into four groups, each with five mice. Control group: received normal saline (0.25ml), second group received lettuce leaf extract (150 mg/kg body weight),third ethanol group received 50% ethanol, and the fourth is Lettuce-Ethanol group received ethanol 50% + lettuce extract (150 mg/kg body weight).Treatment was carry out for 20 days. The results indicated various histological changes in testicular and epididymis tissue induced by ethanol consumption with significant decreased in antioxidant activity levels and increased in lipid peroxidation compared to control group and co-administration with lettuce compensated the damage effects significantly. We concluded that co-treatment mice with lettuce prevent the adverse effect of ethanol toxicity in reproductive male system which proposed the highly antioxidant properties.

KEY WORDS
Lactucasativa L. Testicular antioxidant activity, Testes histopathological changes.

INTRODUCTION

Green leafy vegetables (fresh vegetables) and fruits are important components of a healthy diet [1]. They have dietetic, nutritive value and some may also have the medicinal value [2]. They are providing important vitamins, minerals, and phyto-nutrients [3]. Phyto-nutrients can act as antioxidants, which help to prevent chronic diseases like cancer and cardiovascular diseases [4]. Also many epidemiological studies demonstrated the relationship between dietary habits and disease risk. Because of these potential benefits, many healthy programs around the world have been promoting the consumption of fresh vegetables to prevent diseases [5]. Now days the use of alternative medicine in particular herbal therapies has been propagated among people, because of its economic and no/low side effects [6] Consumption of medicinal plants have the concerned due to the essential antioxidant probable of the phytochemicals that diminish the free-radical and protecting against induced oxidative damage. One of the most common vegetable that consumed as salad in the world is Lettuce (*Lactuca sativa* L.) and that belongs to a member of the composite. Lettuce exhibits healthy properties mainly due to the presence of antioxidant compounds (vitamins C and E, carotenoids, polyphenols) alongside significant fibre content and useful amounts of certain minerals [7]. Therefore it used for treatment of a variety of disorders such as insomnia, neurosis, dry coughs, rheumatic pain [8], and anxiety [9].

The reproductive system, like other body's systems, is affect by many exo and/or endo factors that may have positive or negative effects; for this, many studies are trying to find alternatives like plants to eliminate the effects of negative factors and as a treatment for some cases because of its low cost and the possibility of consumption daily. Alcohol like many factors showed negative effects on many body organs especially the reproductive organs. The essential component of alcoholic beverages is ethanol, the substance responsible for chemical addiction and for a chronic, progressive disease, the alcoholism. Ethanol acts as a toxic component to vigorous organs, acting harmfully on different tissues [10 - 12]. In human, high alcohol consumption is associated with serious damage of spermatogenesis [13]. That reduces sexuality on fertilization. Accordingly, in this study we focused on lettuce as a protective factor and its ability in reducing the negative effects of chronic alcohol consumption.

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MATERIALS AND METHODS

Lettuce leave extraction: Air-dried young Lettuce leaves were ground to powder and the extract prepared using Soxhlet extraction method, ethanol was used as a solvent at a ratio of 1:10 w/v. the extract was evaporated and reconstituted to 10 ml with distilled water and kept at 4 C until used.

Animals: Fifteen mice were housed in animal laboratory of biological department / college of education of pure sciences, Ibn Al-Haitham / University of Baghdad under controlled environmental conditions (12L:12D light cycles; 24C°± temperature). Water and food were given *ad libitum*.

Experimental design: Animals were divided into three groups, 5 each as follows:

1- Control group: received normal slain
2- Lettuce group: received leave lettuce extract (150 mg/kg)
3- Ethanol group: received 50% ethanol orally for 20 days
4- Lettuce-Ethanol group: received ethanol for 20 days + Lettuce extract (150 mg/kg body weight).

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Prepare tissue for histological studies: The right testis fixed in 10% formaldehyde, followed by dehydration with gradual series of alcohol (30 – 100%) and cleared in two changes of xylene, then embedded in paraffin wax for sections. Hematoxylin (Harrison) - Eosin stain was used and sections visualise under light microscope [14].

Tissues preparations for biochemical studies: Animals were cervical decapitation, after dissected testis, the left testis washed with saline solution and homogenize with KH_2PO_4 buffer (100 mM) with EDTA (1 mM, pH 7.5). The homogenize tissue was centrifuged at 13000 g for 20 min at 4 C and supernatant kept for the following biochemical assays.

Determination of lipid peroxidation: Lipid peroxidation was determined according to Guidet and Shah [15]. briefly; 1 ml supernatant of homogenise testes tissue mixed with 1 ml of trichloroacetic acid 17.5 % and 1ml of 0.6% thiobarbituric acid. The mixture was incubated in water bath at 100° for 15 min, after cooling 1ml of 70% TCA was add and left to stand at room temperature for 20 min. samples mixture then centrifuged at 2000 rpm for 15 minutes, and the absorbance was measured at 532 nm.

Determination of catalase activity: CAT enzyme activity was determined followed a method of Aebi [16]. Briefly, 100 µL of supernatant homogenized tissue was added to 1.9 mL of 50 mM phosphate buffer (pH 7.0). Then 1.0 mL of freshly prepared 30 mM H_2O_2 was added to start the reaction. The rate of change was measured at 240 nm.

Determination of glutathione peroxidase activity: Activity of GSH-Pxs was determined according to Godin et al. [17]. The assay mixture contained of phosphate buffer (50 mM, pH 7.0), glutathione (50 mM), 0.1 mL of 30 units/mL glutathione reductase, 0.1 mL of EDTA, 0.1 mL of NADPH (2 mM) and 0.2 mL of samples. The reaction was started by the addition of 0.2 mL of 0.25 mM H_2O_2. The rate of change of absorbance was recorded at 340 nm spectrophotometrically for 3 min.

Determination of superoxide dismutase activity: SOD was determined according to Beyer and Fridovich [18]. Briefly, The assay mixture contained of phosphate buffer (50 mM) with EDTA (0.1 mM, pH 7.8); 0.03 g/mL, L-methionine; 1.41 mg/mL nitroblue tetrazolium chloride (NBT•2HCl); 1% Triton X-100® and 0.4 mL of the samples. The reaction was started by adding 10 µL riboflavin. The mixtures then put in an aluminum box with fluorescent lamps of 20 W for 10 min, and the absorbance was read at 560 nm before and after lighting.

Statistical analysis

Statistical analysis was performed using Minitab 16. All results are presented as mean ± SEM. Significance was accepted at $P \leq 0.05$. The antioxidant treatment data was analysed by one-way analysis of variance (ANOVA) followed by Fisher's least Significant Difference (LSD) test.

RESULTS

Changes in enzymes activity induced by ethanol and lettuce extract: Ethanol treatment significantly decreased the activities of CAT, SOD, glutathione peroxidase and increased in MAD levels as shown in [Table 1] Treatment with lettuce extract significantly improved were the enzyme activities increased whereas MAD level decreased significantly in the tissue homogenate as compared to ethanol treated group.

Testicular histology: The light microscopy results showed normal and intact spermatogenesis epithelium in the control and lettuce treated groups [Figure 1 A and B]. However, different histo pathological alterations were noticed in group treated with ethanol compared to control group. Detected injuries in the seminiferous tubules showed disruption, exfoliation of germinal cells and detachment of different stages of spermatocytes into the lumen [Figure 1 C]. In the other hand, atrophy and degeneration of spermatocytes lining the seminiferous epithelium appeared to be exhausted with appearance of vacuolated tubules. Some of Leydig cells showed nuclear destruction, blood vessels of the interstitial tissue appeared larger and congested. Lettuce + ethanol treated group showed improved in seminiferous tubules structure involving normal spermatogenesis cells along with the interstitial tissue [Figure 1 D].

Epididymis: Epididymis epithelium of control groups as well as lettuce treated group exhibited the typical ciliated epithelial cells (columnar cells in the head and cuboidal cells in the tail) based on basement membrane and enclosed by smooth muscles with connective tissue [Figure 2 A and B]. Epididymis epithelium of ethanol treaded groups showed declined in the epithelial cell height, detachment of epithelial cells from basement membrane and decreased in sperms numbers with occurs of vacuolated epithelial cells and some necrotic cells. Enlargement of interstitial spaces between the tubules also found [Figure 2 C and D], where the epididymis sections of Lettuce + ethanol treated group revealed ameliorated epithelial architecture.

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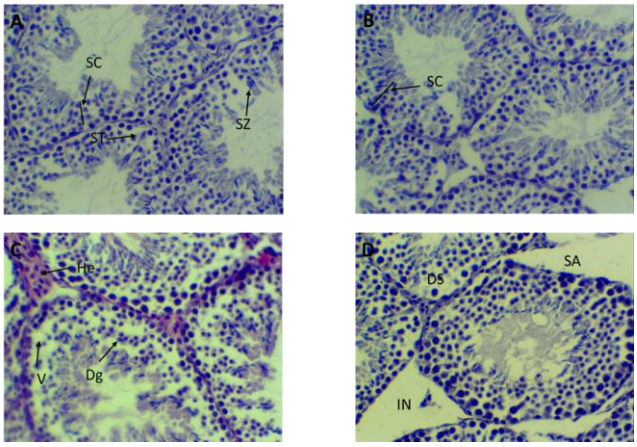

Fig. 1: Crosse section of testes mice showing (A) Control group with normal spermatocytes cells (SC) and sperm (SZ) with Sertoli cell (ST). (B) Testes of mice treated with (150 mg/kg) lettuce extract showing that most seminiferous tubules appeared intact (SC). (C) Testes of mice treated with 50 % of ethanol showing interstitial hemorrhage (He), degeneration (Dg) of some spermatocytes, large vacuole in spermatocytes cytoplasm (V). (D) Testes mice treated with 50% of ethanol + lettuce showing moderated lesion including: disruption and exfoliation in some tubules (DS), seminiferous tubule atrophy (SA), large interstitial space (IN). Stained with H&E, sectioned 400 X.

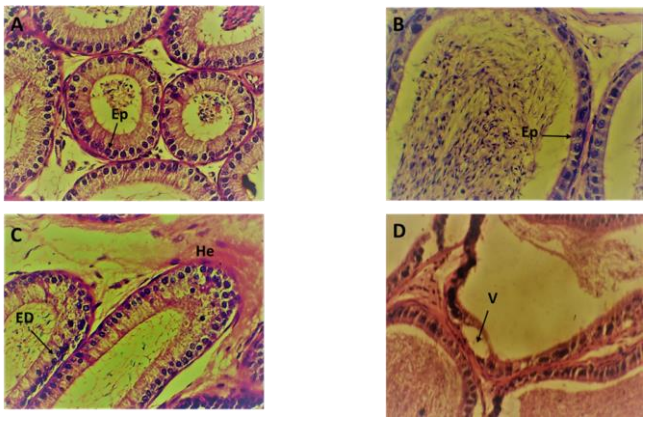

Fig. 2: Cross section of epididymis in mice of (A) Head of control and group treated with lettuce leave extract showing normal columnar epithelium. (B) Tail of control and group treated with lettuce extract showing standard cuboidal cell lining in basement membrane. (C) Head of mice treated with 50% ethanol showing detachment of epithelium, enlargement of interstitial space and hemorrhage. (D) Tail of mice treated with 50% ethanol showing decreased epithelial cell high with appearance of vacuole. Stained with H&E, sectioned 400X.

Table 1: Antioxidant enzymes activity (CAT),(SOD),(MAD) and glutathione peroxidase in homogenised testes tissue of albino mice treated with ethanol extracts of *Lactuca sativa* L

Groups	Dose Mg/kg	SOD U/ml	CAT U/min	MAD µmol/ml	PXs µM/ml
		Treatment for 20 days			
control	Normal saline	A, C 78.9±0.3	A,C44.1±0.3	A, D41.4±1.1	B 27.16±1.5
ethanol	50%	B, C 56.1±0.4	C28.4±0.3	D57.7±1.2	B18.28±1.4
Lettuce	150 mg/kg	C73.2±0.2	C 39.0±0.4	D 45.5±1.7	B 26.50±1.6
Ethanol + lettuce	50% + 150 mg/kg	A, B 67.1±0.4	A36.7±0.6	A51.0±1.5	B 25.04±1.6

A: significance from control group at $P \leq 0.05$, B: significance from ethanol group at $P \leq 0.01$, C: significance from ethanol group at $P \leq 0.001$, D: significance from ethanol group at $P \leq 0.05$.

DISCUSSION

Alcohol consumption has been identified as contributor to chronic tissue injury. This study was carried out to investigate the protective role of Lettuce leave extract in testicular tissue and as well as the antioxidant

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level in mice administrated ethanol. Results observed in the present study suggested that ethanol induced significant decrease in CAT, SOD, and GS-Pxs activity levels, along with increase in MDA level in testis. Furthermore, study results have been shown that ethanol is capable of affecting seminiferous epithelium and altering spermatogenesis. This results were consistent with previous studies which have been determined the effects of ethanol on different animal tissues through metabolic activation to highly reactive substances such as free radicals. Maneesh et al., [19] suggested that histopathological changes observed in rat testes treated with ethanol was due to increase the oxidative stress, as a consequence of free radicals levels increased and a declined in antioxidant defense. Moreover, the other suggested mechanism of the antioxidant activity decreased in testes induced by chronic alcohol feeding, may be due to the enhanced of lipid peroxidation [20]. Histopathological damages in our results is in concert with a study by [21] which suggested that alcohol has a direct and in toxic effect from the decreased in seminiferous tubular function due to negative feedback of fertility hormones; and indirect effect through the HPG axis. The increased in MAD level in this study supported the deleterious effect of alcohol in spermatocytes; spermatozoa are particularly susceptible to oxidative stress-induced damage because their plasma membrane contain large quantities of polyunsaturated fatty acid and their cytoplasm contains low concentration of scavenging enzymes [22] . It has been reported that both acute and chronic consumption of alcohol causes histo-testicular damage, which obstructs spermatogenesis with, decreased in sperm count because of prolonged oxidative stress [23]. On the other hand, mitochondrial function can be impaired by alcohol, thus, promotion of spermatocytes cell death (apoptosis and necrosis) indicated as a result [24].

The interested results in this study was the significant enhanced of lettuce treatment in both histological and antioxidant activities and reduced the lipid peroxidation, this results proposed the free radical scavenging role of the lettuce extract. Oxidative damage can arise as consequence of oxidative stress caused by significant production of reactive oxygen species (ROS), or diminished antioxidant defense mechanisms [25]. Testis is susceptible to oxidative damage than other organs because of its low antioxidant capability and occurrence of polyunsaturated fatty acids in its cell membrane, which are easy targets to oxidative damage by free radicals. Thus, the need for useful green therapeutic agents for enhancing male fertility has been required [26]. Bioactive phytochemicals founds in plants such as saponins, alkaloids and flavonoids have been determined to be valuable therapies for various diseases. For example, flavonoids have been found to be a valuable spectrum of biological activities such as, anti-inflammatory, anti-microbial, anti-proliferative and antioxidant agent [27]. Hefnawy and Ramadan [28] suggested that lettuce extract capable of reduced lipid peroxidation of ethanol induced oxidative damage in liver and testes in rats due to the presence of flavonoids and saponins in the extract. Additionally, the antibictrial and antiviral activities of lettuce methanol extract due to highest total phenolic contents [29]. Moreover, the protective properties of hydro-alcohol extract of *Lactuca sativa* against DNA and protein oxidation with highly value of scavenging activity has been demonstrated [30].

CONCLUSION

Taken together, the results of this study provided new evidence in some potential mechanisms of the effect evince protective role of ethanol extracts of *Lactuca sativa* L to reduce alcohol histopathological impact on male fertility.

CONFLICT OF INTEREST
There is no conflict of interest.

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FINANCIAL DISCLOSURE
None

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