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GRAPE SEED *(VITIS VINIFERA L.)* ALLEVIATE REPRODUCTIVE TOXICITY CAUSED BY LINDANE IN NEW ZEELAND WHITE MALE RABBITS

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Abstract

Natural dietary antioxidants are important for their ability to protect cells from miscellaneous damage. Grape seed (GS) (Vitis vinifera L., Vitacease) is a potent natural antioxidant. The present study aimed to investigate the protective effect of GS against the possible testicular dysfunction caused by Lindane in male rabbits. Eighty sexually mature NZW male rabbits (average BW: 2.150±0.50 kg) were equally divided into four groups, the first served as negative control, the second received Lindane (L) (4 mg/kg body weight 1/ 50 LD 50), the third was supplemented with GS powder (50 g/kg diet), and the fourth received both Lindane and GS (LGS). Doses were given once daily via gavage for 90 consecutive days. The results revealed that, L group induced significant decrease in final body weight, sex organs relative weight, sperm concentration, motility and viability, serum testosterone concentration. Moreover, L altered the histological structure of the testis. Supplementation with GS ameliorated the harmful effects of L, this was also proved histopathologically by the noticeable improvement in the testis tissues. It may be concluded that GS may be promising as a natural therapeutic agent in Lindane -induced reproductive toxicity and oxidative stress in the male rabbit testes.

Key words**:** *Lindane, grape seed, growth performance, semen characteristics*

INTRODUCTION

Chemicals that induce effects by perturbing endocrine systems or mimicking endocrine mediators are collectively described as endocrine disrupting compounds (EDCs). Although pollutants can be very different, chemically and mechanistically, it is appropriate to consider all of the organic and inorganic pollutant classes together and to loosely define them as EDCs because all are known to have disruptive capabilities and they have the potential to interact, additively (Hotchkiss *et al.*, 2008) [24]. Lindane, Hexachlorocyclohexane (HCH), an organochlorine pesticide, impairs testicular functions and fertility, has direct action on reproduction and also carcinogenic properties. Lindane enters the food chain resulting in bioaccumulation in the following order in various tissues: fat, brain, testis, kidney, muscle, lung, heart, spleen, liver and blood (Girima *et al.*, 2011) [20]. Lindane can enter the food chain and lipophylicity facilitates its accumulation in the various tissues of living organisms where, after absorption and distribution, it can easily reach the essential tissues of the reproductive system. Lindane can affect the male reproductive system at either one or multiple sites. These sites include testes, the accessory sex glands, and the central nervous system, including the

neuroendocrine system (Moline *et al*., 2000) [35]. Lindane may directly damage spermatozoa, alter Sertoli cell or Leydig cell function, or disrupt the endocrine function in any stage of hormonal regulation (hormone synthesis, release, storage, transport, and clearance; receptor recognition and binding; thyroid function; and the central nervous system). These mechanisms are described with respect to the effects of insecticides exposure in vitro and in vivo (Saradha *et al*., 2008) [48]. Grape, one of the world's largest fruit crops, with more than 60 million tons harvested per year, is cultivated mainly as *Vitis vinifera* for wine production. Grape (*Vitis vinifera*) seeds are considered as rich sources of poly-phenolic compounds that show antioxidant or antimicrobial effects (Chedea *et al*., 2011) [11]. The antioxidant potential of grape seed extract (GSE) is twenty and fifty fold greater than those of vitamins E and C, respectively (Shi *et al*., 2003) [53].

Therefore, the objective of this work was to investigate the protective effect of GS against the possible testicular dysfunction caused by Lindane in male rabbits.

MATERIALS AND METHODS

The present experiment was carried out at Nuobaria Station, APRI, Agriculture Research Center and Livestock Research Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, New Borg El-Arab, Alexandria, Egypt. Eighty NZW male rabbits aged three months with an average initial body weight of 2.150±0.50 kg were randomly divided into four homogeneous groups with four replicates of 5 animals each (20 rabbits per treatment). Four dietary treatments were formulated as follows: the 1st group was used as control (animals were given orally corn oil); the 2nd group was challenged with 1/50 LD50 of Lindane (L) (Aldrich Chemical Company, Inc. 1001 West Saint Paul Avenue. Milwaukee, Wisconsin 53233 USA), (equal to 4 mg/kg body weight); the 3rd group was given GS (*Vitis vinifera*) (50 g/kg diet); while 4th group supplemented with both chemicals (LGS) (4 mg lindane/kg body weight + 50 g GS/kg diet). Lindane was given orally every day by gelatin capsules, while GS powder was added to the diet during the experimental period for 18 weeks. The proximate chemical compositions of carob pods, the experimental diets and the ingredients used in formulating these diets were determined according to the AOAC (2005) [3]and shown in Table 1.

Table 1.Feed ingredients and chemical composition of experimental diet

Ingredients	g/kg
Barley	220
Soybean meal 44%	200
Wheat Bran	150
Clover Hay	300
Yellow corn	70
Molasses	30
Calcium carbonate	5
Di-calcium phosphate	15
Salt (NaCl)	5
Premix *	3
DL-Methionine	2
Chemical composition (as DM basis g/kg)	
Dry Matter	897.2
Organic Matter	934.8
Crude Protein	174.7
Crude Fiber	137.3
Ether Extract	32.2
Nitrogen Free Extract	590.6
Ash	65.2
DE (Kcal/Kg)**	3,001.01

**Each 3 kilograms of premix contains Vit. A 12000000 IU; Vit. D_3 2000000 IU; Vit. E 10000 mg; Vit K_3 2000 mg; Vit.B_1 1000 mg; Vit. B_2 5000 mg; Vit. B_6 1500 mg; Vit B_{12} 10 mg; Biotin 50 mg; Coline Chloride 250000 mg; Pantothenic acid 10000 mg; Nicotinic acid 30000 mg; Folic acid 1000 mg; Manganese 60000 mg; Zinc 50000 mg; Iron 30000 mg; Copper 10000 mg: Iodine 1000 mg; Selenium 100 mg; Cobalt 100 mg; $CaCO_3$ 3000 mg.*

**DE (Kcal/kg) = 4151-(122*Ash)-(64*Fibre)+(38*Fat)+(23*CP)

All rabbits were housed in double flat galvanized wire batteries (40×50×40 cm) and were kept under the same managerial, hygiene and environmental conditions. Dry matter intake and live BW were recorded weekly on a per cage basis, and feed efficiency was then calculated. Dead rabbits were collected daily and recorded as it occurred.

Semen characteristics

Semen was collected once a week from all animals after 13 weeks of treatment and continued until week 18. Ejaculates were obtained using a teaser doe and an artificial vagina. The volume of each ejaculate was recorded (using a graduated collection tube) after the removal of the gel mass. Determination of seminal initial fructose was carried out directly after collection according to Mann (1948) [31]. Assessments of live, dead and abnormal spermatozoa were performed using an eosin-nigrosine blue staining mixture (Shaffer and Alimquist, 1948) [52]. A weak eosin solution which is described by Smith and Mayer (1955) [54] was used for evaluation of sperm concentration by the improved Neubauer hemocyometer slide. Total sperm output was calculated by multiplying semen volume by semen concentration. Two parameters were calculated to evaluate sperm motility index (SMI): percentage of motile sperm and quality of motility (motility grade). The percentages of motile sperm and motility grade were estimated by visual examination under a microscope (X 10^{10}). Motility was classified as follows: 0 = no movement; grade 1 = twitching, no forward progressive movement (fpm); grade 2 = slow fpm; grade 3 = good fpm; and grade 4 = fast fpm. For calculation of the final test scores, the two motility parameters were combined to yield a sperm motility index (Yousef *et al*, 1996) [60]:

SMI = percentage motile × motility grade

Total number of motile sperm was calculated by multiplying percentage of motile sperm and total sperm outputs.

Serum testosterone determination was performed according to the method adopted by Jaffe and Behrman (1974) [26], by using the coat-A-count technique (radioimmunoassay). Serum triiodothyronine (T3) and thyroxine (T4) levels were determined by using immulite kits (USA) with modifications described by Wells *et al*. (2003) [59]for T3 and Richards *et al*. (1999) [44]for T4.

Carcass traits

At the end of the fattening period, five rabbits were chosen randomly from each treatment; the assigned rabbits were fasted for 16 hours, and dissected after individually weighing. The carcasses were then weighed (without head, heart, spleen, lungs, liver, kidneys and testes) to determine the dressing weight. Liver, kidney and testes as well as dressing weight were expressed relative to live body weight. The whole carcass of each animal was finely ground and all samples were weighed before and after drying overnight at 105 °C. Differences between the two weights represent the moisture content. The dried parts ground well, and the obtained samples were analyzed for protein, fat and ash according to AOAC. (2005) [3].

Statistical analysis

Data were subjected to analyze of variance using GLM procedure of SAS software program package (SAS, 2002) [49]. All percentages were first transformed to arcsine being analyzed to approximate normal distribution before ANOVA according to Steel and Torrie (1981) [55]. Statistical significance of the difference in values of control and treated animals was calculated by F test at 5% significance level.

RESULTS AND DISCUSSIONS

Body weight and feed efficiency

Rabbits were observed for 3-4 hours after the administration of L during the experimental period. It was observed that animals temporarily stopped eating food provided to them after the administration of L. Few clinical symptoms like fatigue, silent, tremor, convulsion, dizziness, occasionally diarrheas, dragging their hind limbs, nasal dripping and trembling were noted in rabbits after oral doses of test chemical. The same findings like trembling, decreased movement, diarrheas were observed in the animals exposed to pesticides in a previous study (Najafi *et al*., 2010) [39]. The changes in body weight throughout the experimental period are summarized in Table 2.

Data showed that treatment with L caused a significant decrease in final live body weight ($P<0.05$), daily weight gain (DWG) ($P<0.05$)

and daily dry matter intake (DMI) (P<0.05) than other groups. However, feed efficiency (FE) was significant (P<0.05) increased due to treatment with L. The results are in agreement with previous studies by Ball and Chabra (1981) [7] suggested that the decline in body weight gain of different species exposed to pesticides may be due to malabsorption of nutrients from the gastrointestinal tract or due to impaired food conversion efficiency in the treated animals.

Table 2.Growth performance of NZW male rabbits treated with Lindane, grape seed, or the combination of both (mean ± SE)

Parameters	Groups				*P* value
	Control	L	GS	LGS	
Initial body weight (kg)	2.155±0.37	2.160±0.81	2.145±0.64	2.140±0.77	0.6584
Final body weight (kg)	3.620±0.28 [a]	2.900±0.32 [c]	3.740±0.51 [a]	3.250±0.18 [b]	0.0029
Live weight gain (g/d)	21.28±0.26 [a]	11.17±0.61 [c]	21.43±0.42 [a]	15.31±0.64 [b]	0.0007
Dry matter intake (g/d)	127.16±9.38[a]	101.69±9.22[c]	122.81±10.82 [a]	110.43±5.01[b]	0.0022
Feed efficiency	5.98±0.36 [c]	9.10±0.22 [a]	5.73±0.19 [c]	7.21±0.27 [b]	0.0036
Mortality %	0	20	0	5	

[abc] Means with different superscript within rows are significantly different (P<0.05).

Mortality was occurred in four test groups during the experimental period. Exposure to pesticides caused reduction in body weight and induced some health problems in animals like e. g. reproductive disorders (Aly *et al*., 2009) [2]. Nersesian *et al*. (2012) [40] reported that decreased feed intake is one of the first responses that rats showed when subjected to certain toxic compounds. Hassan *et al*. (2002) [23] suggested that the hazardous effect of L on feed intake may be due to its effect on the central nerves system (CNS), particularly the hypothalamus which includes feed and water intake center. Also, hyperglycemia which was observed in treated animals probably contributed to the loss of appetite. They suggested that rupture of cells and deformation of tissue may affect the functional activity of the digestive enzymes and this may interfere with digestion, resulting in reduced appetite and growth rate. The decrease in feed intake could be explained by less digestible nutrients which could results in less digestion rate and lower out flow of nutrients from the rumen to the small intestine.

Best (2006) [8] reported that feeding grape seed oil improved body weight of pigs. They suggested that plant products rich in polyphenols may be able to influence the microbial population in the intestine of pigs.

On the other hand, grape residue inclusion up to 30 g/kg did not have any negative effects on growth performance of the broilers (Goñi *et al*., 2007) [21]. Fiesel *et al*. (2014) [19] showed that plant products rich in polyphenols are effective in increasing the gain:feed ratio in growing pigs. Previous studies in rats and broilers have shown that polyphenols are able to cause a shift in the microbial population in the intestinal tract (Viveros *et al*., 2011) [58]. In a study with broilers feeding grape pomace extract or grape seed extract increased counts of beneficial ileal bacteria populations such as *Enterococcus* and decreased counts of potential pathogens such as *Clostridium* were observed (Viveros *et al*., 2011) [58].

Carcass characteristics

Relative organs weight.

Pre-slaughter weight, dressing percentage and relative organs weight to live body weight of rabbits fed the L, GS and LGS are presented in Table 3.

Treatment with L alone caused a significant decrease in dressing percentage (P<0.05) compared to other groups. Treatment with L alone or LGS caused a significant increase in liver and kidney relative weight (P<0.05) compared to control and GS groups (Table 3).

There were no significant differences between control group and GS group in liver, kidney, heart and testes relative weights. The increase in liver and kidney weights in rabbits exposed to L and LGS are in agreement with the results of Elbetieha *et al*. (2001) [15] in rats.
The effect of L, GS and LGS on the carcass chemical composition is presented in Table 3.

Table 3.Carcass weight and meat chemical composition of NZW male rabbit's treated with Lindane, grape seed and the combination of both (mean ± SE)

Parameters	Groups				*P* value
	Control	L	GS	LGS	
Pre slaughter weight, kg	3.200±0.34	2.750±0.48	3.360±0.41	3.090±0.35	0.2554
Dressing (%)	60.26±017^{a}	55.70±0.21^{c}	60.50±0.48^{a}	58.61±0.15^{b}	0.0011
Edible giblets:					
Liver, %	2.34±0.15^{c}	3.29±0.11^{a}	2.41±0.12^{c}	3.06±0.10^{b}	0.0014
Kidney, %	0.72±0.08^{c}	1.13±0.05^{a}	0.78±0.07^{c}	0.99±0.06^{b}	0.0009
Heart, %	0.38±0.02^{b}	0.57±0.03^{a}	0.40±0.03^{b}	0.52±0.05^{a}	0.0043
Testes, %	0.36±0.04^{a}	0.24±0.01^{b}	0.37±0.09^{a}	0.28±0.02^{b}	0.0049
Chemical composition of meat:					
Moisture	69.64±0.31^{c}	72.44±0.22^{a}	69.23±0.18^{c}	70.46±0.33^{b}	0.0008
Crude protein	20.68±0.21^{a}	19.33±0.27^{b}	20.46±0.16^{a}	19.73±0.11^{b}	0.0011
Ether extract	5.33±0.14^{b}	6.05±0.16^{a}	4.96±0.11^{b}	5.88±0.10^{a}	0.0015
Ash	1.45±0.08	1.65±0.10	1.47±0.11	1.73±0.24	0.3365

^{abc} Means with different superscript within rows are significantly different (P<0.05).

Data showed that treatment with L and LGS caused a significant increase in moisture and fat content (P<0.05) and decrease in CP content compared to the other groups. Gupta *et al*. (1983) [22] reported that the inhibiting effect of pesticide on protein synthesis was dose-dependent. Cecil *et al*. (1974) [10] found that liver's lipid content can increased significantly (P<0.05) when female rats and quail were treated with Malathion. The present results do indicate that body composition had been altered by pesticide treatment and resulted in enhanced fat deposition and prevented fat mobilization.
In this study, higher protein and lower lipid levels were found in the body of rabbits fed diet containing GS. The higher protein level of GS group is probably related to the decreased crude lipid levels which the later might be due to GS effects on lipid metabolism. It was found that GS could repress intestinal lipid absorption, chylomicron secretion by the intestine, very low density lipoprotein secretion by the liver (Ngamukote *et al*., 2011) [41], inhibit intestinal lipoprotein secretion (Vidal et al., 2005) [57], inhibit cellular cholesterol uptake and 5-lipoxygenase activity (Leifert and Abeywardena, 2008) [30], and stimulate serum ability to induce efflux of cellular cholesterol (Senault *et al*., 2000) [51]. Tekeli *et al*. (2014) [56] showed that abdominal fat weight tended to decrease in the groups fed 5 and 10 g/kg GS oil. Moreno *et al*. (2003) [37] reported that GS extract limited fat deposition in adipose tissue by inhibition of the fat metabolizing pancreatic enzymes, lipoprotein and hormone-sensitive lipase and hence controlled obesity.
The weights of the testis, epididymis, seminal vesicles and ventral prostate decreased in rats exposed to methoxychlor (Latchoumycandane and Mathur, 2002) [29]. The activities of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase decreased in testes. The levels of hydrogen peroxide generation (H_2O_2) and lipid peroxidation increased in testis of the rats after methoxychlor exposure. DDT and some organic solvents lead to decreased fertility and altered sperm counts. The DDT can also delay puberty (Moreira and Wolff, 2003) [36]. The effects of high exposure to Tetrachloro-dibenzo-p-dioxin (TCDD) and "TCDD-like" compounds on important sites for development and reproduction have also

been recognized by Eskenazi and Kimmel (1995) [18]. Profenofos exerted toxic effects on testicular tissues and disrupting the testicular function in treated animals which was associated with significant reduction in testes weight (El-Kashoury, 2009) [16].

Semen characteristics

Semen characteristics are important in determining the fertility of rabbit bucks. Non-genetic factors such as stress, nutrition, age and management are believed to influence semen characteristics and subsequently buck fertility. Rabbits quickly adapted themselves to semen collection procedure employed. The majority of the treated animals, especially those treated with combination of L and GS indicated reduced libido. Data on semen ejaculate volume and sperm quality parameters of rabbits treated with L, GS, and the combination of both are presented in Table 4.

Table 4. Semen characteristics of NZW male rabbit treated with Lindane, grape seed and the combination of both (mean + SE)

Parameters	Groups				*P* Value
	Control	L	GS	LGS	
Semen volume (ml)	0.95±0.01 ^{b}	0.67±0.02 ^{d}	1.00±0.02 ^{a}	0.82±0.04 ^{c}	0.0031
Sperm Concentration (X 10^{6}/ml)	400.33±12.37^{b}	247.33±15.67^{d}	460.10±23.55^{a}	370.44±25.18^{c}	0.0044
Total sperm output (X 10^{6} sperm)	380.31±22.46^{b}	165.71±19.33^{d}	460.10±18.28^{a}	303.76±31.27^{c}	0.0028
Sperm motility (%)	85.25±0.48^{a}	61.50±0.83^{c}	85.75±0.77^{a}	75.50±1.06^{b}	0.0007
Sperm motility grade	3.88±0.06^{a}	2.44±0.07^{c}	3.79±0.08^{a}	3.05±0.08^{b}	0.0039
Sperm motility index (SMI)	3.31±0.09^{a}	1.50±0.10^{c}	3.25±0.08^{a}	2.30±0.05^{b}	0.0023
Total motile sperm per ejaculate	324.21±11.52^{b}	101.91±14.88^{d}	394.54±9.84^{a}	229.34±12.31^{c}	0.0001
Abnormal sperm (%)	11.41±0.38^{c}	29.25±0.47^{a}	10.77±0.41^{c}	19.35±0.33^{b}	0.0026
Dead sperm (%)	7.33±0.10 ^{c}	18.55±0.24 ^{a}	6.98±0.15 ^{c}	12.66±0.11 ^{b}	0.0024
Semen initial fructose (mg/100ml)	112.66±7.13 ^{c}	261.38±16.83 ^{a}	113.19±8.15 ^{c}	187.92±9.03 ^{b}	0.0019

^{abc} Means with different superscript within rows are significantly different(P<0.05).

Data showed that treatment with L caused a significant decrease in semen ejaculate volume, sperm concentration, total sperm output, sperm motility, sperm motility grad, sperm motility index (SMI) and total motile sperm per ejaculate (P<0.05) compared to other groups. While treatment with L caused a significant increase in percentage of abnormal sperm, dead sperm and semen initial fructose (P<0.05) compared to other groups. These results agreed with previous studies showing reduced semen quality in men occupationally exposed to various pesticides. Azoospermia, testicular dysfunction and sterility were also noted in men chronically exposed to dibromochloropropane, DBCP (Balash *et al*., 1987) [6]. Salem *et al*. (1988) [46] also reported that dimethoate and deltamethrin reduced libido, ejaculate volume, sperm concentration and total epididymal sperm counts and deferens sperm concentration, and caused slight to severe hypospermatogenesis of the male rabbits. Motility is involved in defining the ability of the spermatozoa to ascend the reproductive tract to the site of fertilization, as well as the act of fertilization itself, particularly regarding the penetration of the vestments surrounding the oocyte,

including the cumulus oophorus and zona pellucida. Considering the significance of sperm motility, it is not surprising that this criterion of sperm function has assumed a central role in the routine clinical diagnosis of male fertility (Aitken, 1990). This author suggested that pesticide's disruption of reproductive processes might be partly due to adverse effects on sperm cell function. Pesticides had a damaging effect on spermatogenesis and this disturbance was due to the lack of local testosterone. The effects of pesticides on spermatogenesis may be mediated through their effects on hormonal balance. Krause (1977) [27] also reported that Malathion and dichlorvos decreased fertility, spermatogenesis and increased sperm abnormalities in rats, and these effects were attributed to a direct cytotoxic action on the testes. The presented data showed a significant increase in semen initial fructose. The fructose formation by the accessory glands is dependent upon the secretion of testosterone by the testes (Atterwill and Steele, 1987) [4]. Lindane, an organochlorine pesticide, impairs testicular functions and fertility. Lindane has direct action on reproduction and also carcinogenic properties. Treatment with 1-40 mg of L/kg body weight disrupted testicular morphology, decreased spermatogenesis and impaired reproductive performances in males (Page *et al*., 2002) [43].

Sperm motility parameters were increased in this study by supplementing the diet with GP. In addition, GP antioxidants may offer protection against the damaging effect of leukocyte-derived reactive oxygen species on sperm movement (Baker *et al*., 1996) [5]. It could be assumed that the observed increases in sperm motility after the GP treatments could partly be attributed to the concomitant induction in semen fructose (Yousef, *et al*., 2003) [61].The positive effects of dietary GP on both sperm count and sperm motility and the reduced percentage of dead sperm could be linked to the antioxidative properties of GP (Murthy *et al*., 2002) [38]. It had been suggested that the morphology and the motility of sperm cells would be preserved by binding antioxidants to endo-peroxides (Marin-Guzman *et al*., 2000) [32]. Recently, Eid *et al*. (2006) [14] found that a higher antioxidant intake was associated with greater sperm numbers and motility.

Serum hormones

Data in Table 5 demonstrates testosterone, T3 and T4 concentrations in blood plasma. The GS group had significantly greater testosterone, T3 and T4 concentrations ($P<0.05$) in blood plasma than the other groups. The significant decrease of testosterone level may be a result of direct damage of dicofol on leydig cells, which are the main site of testicular androgen biosynthesis.

Table 5. Serum hormones of NZW male rabbit treated with Lindane, grape seed and their combination (mean ±SE)

Parameters	Groups				*P* values
	Control	L	GS	LGS	
Testosterone (ng/ml)	2.06±0.15 [b]	0.76±0.21 [d]	3.78±0.18 [a]	1.22±0.09 [c]	0.0001
T3, ng/ml	5.45±0.16 [b]	2.28±0.18 [d]	6.49±0.12 [a]	3.33±0.14 [c]	0.0001
T4, ng/ml	22.82±0.32 [b]	10.71±0.41 [d]	25.58±0.11 [a]	14.11±0.16 [c]	0.0001

[abc] Means with different superscript within rows are significantly different ($P<0.05$).

Results of the present work agreed with those found by Choudhary and Joshi (2003) [12], who noted that T level was significantly decreased in male rats exposed to organochlorine pesticides at different doses, i.e. DDT, PCB-126 and 153, methoxychlor, DDT, endosulfan. El- Kashoury *et al*. (2003) [17] described similar changes in T4 and T3 levels after dicofol exposure at lower and higherdoses. They also reported that the decrease in T4 levels may be a result of iodine deficiency that causes the gland fail to synthesize T4 and therefore hypothyrodism occurs. Hotz *et al*. (1997) [25] also reported that, pesticide increased deiodination and biliary excretion of thyroid hormone T4 which led to increased rate of T4 elimination from the blood. Some insecticides, herbicides

and fungicides disrupt endocrine system. Thyroid disruptors affect through different mechanisms (Boas *et al*., 2006) [9]. It has been shown that some thyroid disruptors inhibit thyroperoxidase; thereby they change ability of follicular cell in producing T4 and then T3, even at sufficient iodine concentration. Animal studies have revealed that amitrol (herbicide), ethylenethiourea (fungicide), Mancozeb (fungicide); bean isoflavones and benzophenone 2 inhibit production of thyroperoxidase and prevent thyroglobulin synthesis. Therefore, T3 and T4 synthesis is reduced (Miller *et al*., 2009) [34]. Many chemical compounds have high structural similarity to thyroxin and T3 thereby they disrupt the binding of thyroid hormones to their receptors or transferring proteins. This case can in turn result in subclinical hypothyroidism, which is randomly diagnosed in adults due to its mild symptoms. The results of this study suggested that exposure to organophosphorus and organochlorine pesticides, which are the most widely used pesticides, may changes serum level of thyroid stimulating hormone (TSH), total T4 and total T3 in human in the long-term. In another study, the relationship between urine concentration of dialkyl phosphate and TSH and thyroid hormone levels were examined among farmers (Lacasana *et al*., 2010) [28].

Histological Parameters

The histological structure of testes in control animals consisted of seminiferous tubules with rounded /oval shaped and spermatocytes were also noted in scattered position throughout the tubules. Sertoli cells were also present inside the seminiferous tubules along with leydig's cells within interstitial space (Zidan, 2009) [62].

In the present study histological structure of testis in the control animals showed normalsize seminiferous tubules containing different types of spermatogenic cells in different stages of spermatogonia and spermatocytes with appearance of normal and narrow interstitial space and leydig's cells of normal size were also present in the interstitial space (Fig. A).

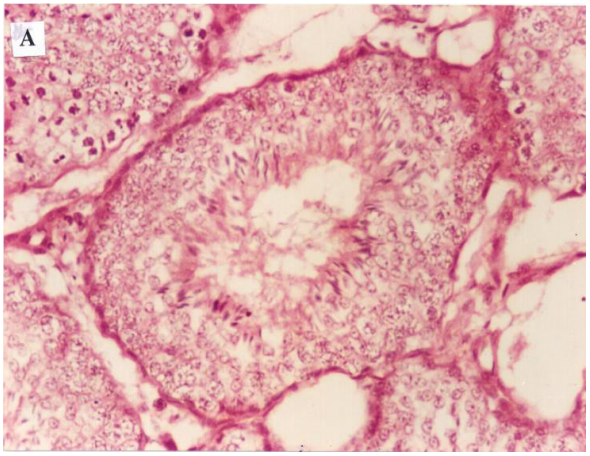

Fig. A. Photomicrograph of testes of control rabbit showing normal semineferous tubules.

However histopathological changes were observed in the animals exposed to L for 90 days. Tumor-like mass was present in few tubules along with other changes like e. g. vacuolation in seminiferous tubules and suppressed number of leydig's cells. The seminiferous tubules were found with abnormal size and shape indicated in (Fig. B).

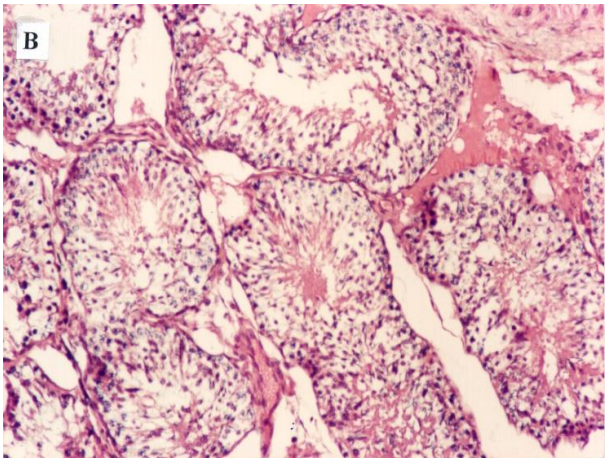

Fig. B. Photomicrograph of testes of rabbit treated with Lindane showing degenerative changes of spermatocytes, edema between some semineferous tubules and anest of spermatogenesis.

Moreover, our investigations revealed that, the size of seminiferous tubules were further reduced as well as condensed interstitial space. The number of spermatogenic cells was regressed and leydig's cells have been either hypertrophied or eliminated. Destruction in leydig's cells, disrupted the functioning of the testes to release testosterone hormone for the development of spermatogenic cells (Saunders, 2003) [50]. In the present study

hypertrophyin interstitial spacewas clearly seen and no spermatocytes were found in the seminiferous tubules. The leydig's cells were totally regressed or vanished, that shows drastic endocrine disrupting effects of L on leydig's cells and testes (Fig. B). Exposure of pesticides exhibited pathological changes in testicular tissues and this alteration occurred as antiandrogenic effects (Dallegrave *et al*., 2007) [13].

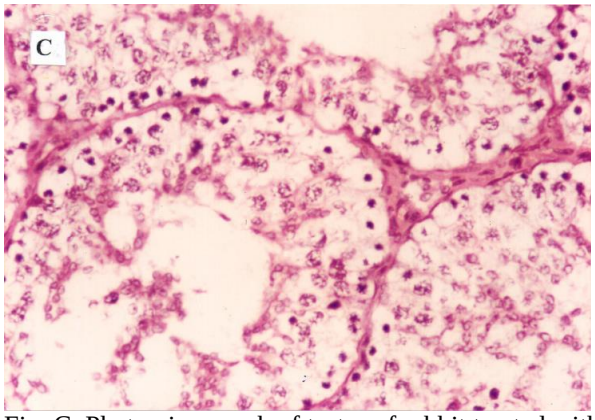

Fig. C. Photomicrograph of testes of rabbit treated with grape seed showing normal semineferous tubules.

The results indicated that the toxicity of L on testes depends on the dose. The changes predominantly consisted of moderate edema, congestion, damage to Sertoli cells and germ cells, along with the accumulation of cellular debris and presence of giant cells in the lumen of a few seminiferous tubules. More recently, several studies have investigated the effect of mixtures of endocrine disrupting compounds (EDCs) on the developing rodent testis and its functions, and have shown that combinations of the compounds e.g. anti-androgenic EDCs, exert major effects at doses at which the individual EDCs have no significant effect (Rider *et al*., 2009) [45]. In adult Wistar rats orally treated with pirimiphos-methyl (41.67, 62.5 or 125 mg/kg) for 90 days, a decrease in relative testis and epidiydimis weights and intra-testicular cholesterol level were recorded. Whereas a decrease in serum total protein, sperm density and motility, fertility and parturition indices and pups sex-ratio (M/F) were recorded in animals treated with 125 mg/Kg of pirimiphos methyl. Histological findings also indicated enlargement of interstitial space, inhibition of spermatogenesis, rarefaction of Leydig cells and edema in testes of treated rats (Ngoula *et al*., 2007) [42].

The role of GS in male fertility is very well documented. Supplementation of GS has been reported to restore the spermatogenic process and thus fertility damage by toxic heavy metals (Sallam *et al*. 2005) [47], reduces oxidative stress-related effects on spermatogenesis in Cd-treated swiss mice (Acharya *et al*. 2008) [1].

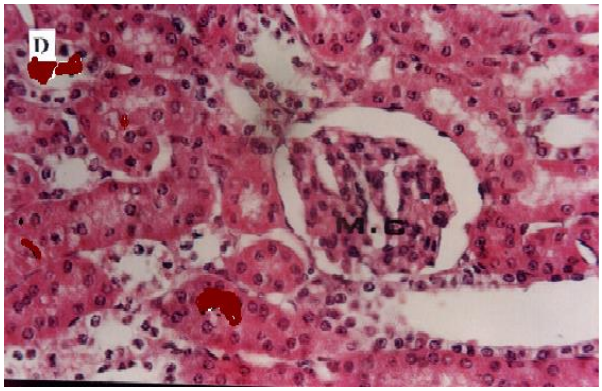

Fig. D. Photomicrograph of testes of rabbit treated with the combination of Lindane and grape seed showing degenerative changes and necrosis semineferous tubules. spermatogenesis

In this study, L induced marked degenerative changes in caput and cauda epididymis and the vas deferens, but these changes were prevented with GS supplementation in combination with L. It has been suggested that the degenerative changes in the epididymis and vas deferens caused a decreased bioavailability and production of androgens (Mennela and Jones 1980) [33]. Thus L may modulate androgen levels in the blood by acting on androgen-producing cells or through the hypothalamo-hypophysial-gonadal axis. Since the epididymal epithelium structure, function and spermatozoa maturation are all androgen-dependent, hence the observed degenerative changes in the epididymis caused by L exposure may be due to low androgen levels in the serum. Recovery of epididymal structure after GS supplementation may indicate the restoration of androgen synthesis. All these observations suggest that L has androgen-antagonistic functions in male Mus musculus. Since administration of GS showed normal

histoarchitectural features in the epididymis and vas deferens, this indicates that GS has antioxidative and protective roles against L toxicity.

CONCLUSIONS

The rabbits group whose diet included Lindane registered a decline in the final body weight, sex organs relative weight, sperm concentration, motility and viability, serum testosterone concentration. Also, the diet including Lindane altered the histological structure of the testis.

The inclusion of grape seeds (GS) in the diet ameliorated the harmful effects of Lindane. This was histopathologically demonstrated by the noticeable improvement in the testis tissues.

Therefore, the use of grape seeds in the diet increased the quality of the rabbit semen probably due to the physiological and antioxidant effects.

As a final conclusion, in the male rabbit testes, grape seeds may be a natural therapeutic agent in Lindane-induced reproductive toxicity and oxidative stress.

More detailed studies regarding this particular aspect are required.

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