

Efficacy of *Curculigo orchioides* in Deltamethrin Induced Reproductive System Impairment in Male Wistar Rats

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Abstract

Aim: To evaluate the ameliorating effect of *Curculigo orchioides* (CO) against deltamethrin (DLM) induced reproductive toxicity in male rats. **Materials and Methods:** A total of 42 adult male Wistar rats were divided into seven groups of six rats each. Group A (control) received normal diet and water *ad-libitum*, Group B (exposure) received DLM (2 mg/kg bw), orally in dimethylsulphoxide for 60 days, Group C (Treatment 1) received CO (100 mg/kg bw), orally dissolved in distilled water, Group D (Treatment 2) received CO at the dose of 200 mg/kg bw dissolved in distilled water, Group E (Combination 1) received DLM at the dose of 2 mg/kg with CO (100 mg/kg bw), Group F (Combination 2) received DLM at the dose of 2 mg/kg with CO (200 mg/kg bw), and Group G (metabolized) received DLM at the dose of 2 mg/kg bw for 60 days and DLM was left to metabolized for 60 days. **Results and Discussion:** DLM exposure caused significant reduction in body weight, reproductive organ weight, sperm head counts, sperm motility, reduced glutathione (GSH), catalase, superoxide dismutase (SOD), GSH-S-transferase (GST), GSH peroxidase (GPx), GSH reductase (GR), testosterone (T), follicles stimulating hormone (FSH), luteinizing hormone (LH) and steroidogenic enzyme 3 β hydroxysteroid dehydrogenase (3 β HSD), and 17 β HSD. DLM exposure also showed increase in sperm abnormalities and level of lipid peroxidation. The treatment with CO improved the body weight, reproductive organ weight, sperm head count, sperm motility, and sperm abnormality. CO treatment also increased the level of GSH, SOD, GST, GPx, GR, T, FSH, LH and steroidogenic enzyme 3 β HSD, and 17 β HSD. Histological findings indicated vacuolation in lumen, disorganization of leydig cells and sertoli cells and inhibition of spermatogenesis in testicular tissue on DLM exposure, whereas CO treatment recovered normal histoarchitecture. **Conclusion:** The findings indicated that DLM damaged the reproductive system of male Wistar rats. CO treatment efficiently improved the reproductive system chiefly by increasing the level of important steroidogenic enzymes and hormones.

Key words: *Curculigo orchioides*, deltamethrin, oxidative stress, reproductive toxicity

INTRODUCTION

The incidences of infertility are estimated to be about 13-18% in the human population, regardless of geographical area, race, and ethnic group.^[1-5] Male partner is responsible for almost half of the infertile cases. Male infertility refers to the inability of a male to establish pregnancy in a fertile female. The main cause of male infertility are oligospermia (<20 million sperm per milliliter), asthenospermia (reduction of spermatozoon motility), teratozoospermia (high percentage of morphologically abnormal spermatozoa in ejaculates), and azoospermia (complete absence of sperm in the ejaculate). These causes accounts for 20-25% of male infertility.^[6,7]

Exposure to xenobiotics is a proven factor in impairment of reproductive function in animals including humans. Pesticides are persistent organic pollutants, which causes reproductive toxicity in human. The chronic exposure of even small no observed effect limit doses of pesticides alters reproductive system physiology which may lead to

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male infertility. Pyrethroids have become extremely popular insecticides mainly due to their relatively low mammalian toxicity and rapid biodegradability. Deltamethrin (DLM) is a synthetic pyrethroid used extensively in pest control programs. DLM is used in eliminating and preventing a variety of household pests, especially spiders, fleas, ticks, carpenter ants, carpenter bees, cockroaches, bedbugs, and primary ingredient in ant chalk. Although initially considered to be safe, recent reports showed its reproductive toxicity in mammalian and non-mammalian laboratory and wildlife animal species.^[8-10] The mild reproductive toxic effects of pyrethroids on humans were largely due to occupational exposure, however, no long-term or persistent effects have been reported.

The optimum level of testosterone (T) is essentially required for producing adequate number of healthy sperms needed for fertility. This hormone is responsible for functional germ cells as well as maturation of sperms. Testosterone is synthesized from cholesterol in the steroidogenic cells. Testosterone biosynthesis essentially requires the transport of cholesterol from the cytoplasm to the inner mitochondrial membrane where cytochrome P450 side chain cleavage enzyme cleaves its side chain and converts it to pregnenolone. Two microsomal enzymes, 3β hydroxysteroid dehydrogenase (3β HSD), and 17β HSD further regulates the biosynthesis of testosterone from pregnenolone. 3β HSD converts pregnenolone to progesterone which is later converted to androstenediol. Androstenediol is converted to testosterone by 17β HSD. The transport of cholesterol from outer to inner mitochondrial membrane is a rate-limiting step in testosterone biosynthesis. The hydrophilic environment of inner membranous space hinders the transfer of cholesterol from outer to inner membrane. The transport of cholesterol from outer to inner mitochondrial membrane is facilitated by steroidogenic acute regulatory protein (StAR). Increase in expression of StAR protein is also known to accelerate testosterone biosynthesis.

Reports of DLM toxicity on testosterone biosynthesis are not available. Hence, it was planned to assess the effect of DLM on reproductive system of male Wistar rats with a focus on role of StAR protein, steroidogenic enzymes 3β HSD, and 17β HSD in testosterone biosynthesis.

A number of allopathic drugs (such as sildenafil, cialis, or levitra) have been developed to improve male sexual function impaired due to emotional, occupational and environmental stress, but side effects associated with these drugs limit their utility.^[11] Hence, there has been a persistent demand for safe and effective aphrodisiac drugs, especially of herbal origin. "Vajikaran" is a branch of Ayurvedic therapeutics for potentiating male fertility and improving sexual performance. As per literature survey approximately 13,000 plants have been studied during the last 5-year period for treating men for improving potency and alleviating sexual dysfunction.

Some of the reproductive system enhancer plants are *Phoenix dactylifera*, *Fadogia agrestis*, *Chione venosa*, *Lepidium meyenii*, *Moringa oleifera*, *Abrus precatorius*, *Asparagus racemosus*, *Hygrophila spinosa*, *Lophira lanceolat*, *Tibullus alatus*, *Eurycoma longifolia*, *Eriosema kraussianum*, *Butea frondosa*, *Butea superb*, *Mucuna pruriens*, *Withania somnifera*, *Asteracantha longifolia*, and *Tribulus terrestris*.^[12,13]

Curculigo orchioides (CO) Gaertn. (family Amaryllidaceae), *Kali Musli*, is used as a male sexual tonic in Ayurvedic system of medicine. The plant possesses potent antioxidant and adaptogenic properties. The plant is reported to possess estrogenic,^[14] pendiculatory,^[15] hepatoprotective,^[16] immunostimulant,^[17,18] and antioxidant^[19] activities.

The present work is planned to study the effect of hydroalcoholic extract of CO on DLM induced impairment of reproductive system in male Wistar rats, with emphasis on steroidogenesis.

MATERIALS AND METHODS

Animals

Male Wistar rats, weighing about 200-250 g, were used for experimental work. Animals were obtained from Defense Research and Development Establishment, Gwalior and were kept for acclimatization in the animal house at ambient temperature of 25°C and 45-55% relative humidity, with 12 h each of dark and light cycles. Animals were fed pellet diet and water *ad-libitum*. All animal experiments were performed at the consent of the Animal Ethical committee (BU/pharm/IAEC/13/21).

Chemicals

Technical grade pure DLM (98.5%) was gifted by Gharda Chemicals Ltd., Mumbai. All others chemical were of AR grade and purchased locally.

Plant extraction

Authenticated roots of CO were obtained from the Shree Baidyanath Ayurved Bhawan Ltd., Jhansi and pulverized to obtain coarse powder. Powdered material was defatted with hexane through mechanical stirring process for 6 h. The residue was dried and stored overnight at room temperature. The dried material was extracted with 70% ethanol with constant mechanical stirring for 6-8 h. Ethanolic extract so obtained was centrifuged at 4000 rpm for 30 min at 5°C temperature for removal of any particulate material. The filtrate was concentrated under vacuum at room temperature followed by lyophilization in Martin Christ alpha 1-2 LD plus lyophilizer to get the dried extract.

Experimental schedule

The rats were divided into seven groups of six each as per treatment schedule given below. DLM and CO were administered orally by gavaging in dimethylsulphoxide and distilled water, respectively, for 60 days. Dose of CO was decided as per previous reference.^[20]

Treatment schedule

Group A	Vehicle control	Normal diet and water for 60 days
Group B	Exposure	DLM 2 mg/kg bw for 60 days
Group C	Treatment 1	CO (100 mg/kg bw) for 60 days
Group D	Treatment 2	CO (200 mg/kg bw) for 60 days
Group E	Combination 1	DLM (2 mg/kg bw) along with CO (100 mg/kg bw) for 60 days
Group F	Combination 2	DLM (2 mg/kg bw) along with CO (200 mg/kg bw) for 60 days
Group G	Metabolized	DLM (2 mg/kg bw) allowed to metabolized for 1 month after 60 days exposure

DLM: Deltamethrin, CO: *Curculigo orchioides*

At the end of the experiment, rats were sacrificed, both the testis, epididymis prostate and seminal vesicles were removed and weighted. One testis was used for sperm head counts and the epididymis was used for sperm motility and sperm morphology studies. Other testis was used for estimation of lipid peroxidation (LPO), enzymatic and non-enzymatic antioxidants, and steroidogenic enzymes. A part of testis was kept in 10% formaldehyde for histological studies. Blood samples were taken from heart, and serum was separated for estimation of various reproductive hormones.

Biochemical estimations

Preparation of tissue homogenate

Testis was homogenized with 10% (w/v) homogenizing buffer (0.1 M phosphate buffers, pH 7.4 + 150 mM KCl). A part of this 10% homogenate was used for LPO and glutathione (GSH) estimations. The remaining part of 10% homogenate was centrifuged at 9000 rpm for 20 min to get supernatant (S) fraction which was used for superoxide dismutase (SOD), catalase (CAT), GSH peroxidase (GPx), GSH reductase (GR), and GSH-S-transferase (GST) estimations.

LPO

LPO was estimated by the method of Ohkawa *et al.*^[21] 1 ml of 10% homogenate was incubated at 37°C for 10 min and 1 ml of 10% (w/v) chilled trichloroacetic acid (TCA) was added to it. The mixture was centrifuged at 2500 rpm for 15 min at room temperature. 1 ml of 0.67% thiobarbituric acid was added to 1 ml of supernatant and kept in boiling water bath for 10-15 min. The tubes were cooled under tap water, followed by addition of 1 ml of distilled water. Absorbance was recorded at 530 nm, and the results were expressed as nmole MDA/h/g tissue.

Reduced GSH

GSH was estimated by the method described by Ellman.^[22] 1 ml of 10% homogenate was mixed with 1 ml of 5% TCA (w/v), the mixture was allowed to stand for 30 min and centrifuged at 2500 rpm for 15 min. 0.5 ml of supernatant was taken and 2.5 ml of 5' 5'-dithionitrobenzoic acid was added, mixed thoroughly and absorbance was recorded at 412 nm. The results were expressed as μ mole/g tissue.

Enzymatic antioxidant

CAT

CAT activity was estimated by the method of Sinha.^[23] 1 ml of phosphate buffer and 0.4 ml water was added to 0.1 ml of supernatant (S). Reaction was started by adding 0.5 ml H₂O₂ and mixture was incubated at 37°C for 1 min. Reaction was stopped by adding 2 ml of dichromate: Acetic acid reagent and kept at boiling water bath for 15 min. The mixture was cooled and absorbance was read at 570 nm. CAT activity was calculated in terms of μ mole/min/mg.

SOD

SOD activity was estimated by the method of Kakkar *et al.*^[24] 650 μ l of sodium pyrophosphate buffer, 50 μ l phenazine methasulfate, 150 μ l of nitroblue tetrazolium chloride, and 100 μ l nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) were added to 50 μ l of supernatant. The mixture was vortexed thoroughly, incubated for 90 s and 500 μ l glacial acetic acid was added to stop the reaction. 2.0 ml of n-butanol was added to the mixture, vortexed thoroughly and kept at room temperature for 10 min. Absorbance was measured at 560 nm and the results were expressed as μ mole/min/mg protein.

GST

GST was estimated as per method of Habig *et al.*^[25] The reaction mixture consisting of 1.425 ml phosphate buffer (0.1 M, pH 6.5) 1.475 ml GSH (1.0 mM), 20 μ l 1-chloro-2,4-dinitrobenzene (CDNB, 1 mM), and 60 μ l water were added to 20 μ l of supernatant to give 3.0 ml of reaction mixture. Absorbance was recorded at 340 nm and the GST activity

was calculated as μ moles CDNB conjugate formed/min/mg protein.

GPx

GPx was estimated by the method of Rotruck *et al.*^[26] 0.4 ml Tris HCl buffer, 0.2 ml GSH, 0.1 ml water, and 0.2 ml H_2O_2 were added to 0.1 ml of supernatant. The mixture was incubated at 37°C for 15 min and 0.5 ml TCA (10%) was added. The mixture was centrifuged at 2000 rpm for 15 min, 0.5 ml of supernatant was taken and 2 ml disodium hydrogen phosphate buffer and 0.5 ml Ellman's reagent were added and the absorbance was read at 420 nm. The results were expressed as nmole/min/mg protein.

GR

GR was estimated by the method of Carlberg and Mannervik.^[27] 2.5 ml buffer, 0.2 ml NADPH, 0.2 ml GSSG and 0.1 ml "supernatant" were mixed and allowed to stand for 30 s. Absorbance was recorded at 340 nm for 3 min at 30 s intervals. GR activity was calculated regarding nmoles/min/mg protein.

Sperm parameters

Sperm head counts

Sperm head counts were performed using hemocytometer as described by Choi *et al.*^[28] with necessary modifications. The testes were separated; weighed and tunica albuginea (outer covering) was removed. The testes were minced and homogenized in 0.9% NaCl and 0.05% triton X solution for 2 min at 8000 rpm. 10-15 μ l of homogenate was placed on hemocytometer. After 5 min sperm heads were counted in red blood cell chamber at $\times 40$ magnification.

Sperm abnormality

A segment of epididymis was minced in 1 ml of 0.9% saline with the help of razor and 1 ml of 10% neutral buffer saline was added. The suspension was diluted with water to suitable volume for performing the assay. 1-2 ml of eosin (1%) was added to 20 ml of above suspension and incubated at room temperature for 1 h. One drop of suspension was taken on slide and a smear was prepared. The slides were viewed under light microscope at $\times 40$ magnification for evaluation of head and tail abnormalities. A total of 200 sperms were examined on each slide and head and tail abnormalities were expressed as percentage.^[29]

Reproductive hormones analysis

Serum testosterone, luteinizing hormone (LH), and follicles stimulating hormones (FSHs) were assayed by Qayee-Bio Life Science ELISA kits.

Steroidogenic enzymes

3 β HSD and 17 β HSD were estimated by Cusabio ELISA kits.

Statistical analysis

Experimental results were expressed as a mean \pm standard error of mean. The intergroup variation was measured by one-way analysis of variance followed by Dunett's test. A statistical significance was considered at $P < 0.05$. The statistical analysis was performed using Graph Pad In Stat Software Inc., V. 3.06 (San Diego, USA).

RESULTS

Body weight

The body weight of rats was non-significantly ($P > 0.05$) increased in the control Group A (3.4%) at day 60 in comparison to day 1. A significant decrease ($P < 0.01$) in body weight was observed at day 60 in DLM exposed Group B (12.41%) and in metabolized Group G (10.61%), respectively, as compared to day 1. At day 60 non-significant ($P > 0.05$) increase in body weight was found in Group C (3.755%) and significant ($P < 0.01$) increase in Group D (4.86%), as compared to day 1. Significant ($P < 0.01$) decrease in body weight in the combination Group E (4.12%) and non-significant ($P > 0.05$) decrease in Group F (2.31%) have been observed at day 60 compared to day 01 [Table 1].

Sex organ weight

Table 2 showed that the weight of testis were significantly ($P < 0.01$) decreased in Group B (26.84%), Group G (16.77%) and in Group E ($P > 0.05$, 5.36%) as compared to Group A. On the other hand, non-significant ($P > 0.05$) increase in weight of testis was observed in Group C (4.02%), Group D (7.38%), and Group F (1.34%) compared with the control group. A significant ($P < 0.01$) increase in weight of testis was seen in Group E (29.44%), Group F (38.53%) and non-significant ($P > 0.05$) increase in Group G (13.67%) as compared to Group A. The weight of prostate and seminal vesicle were significantly ($P < 0.01$) decreased in Group B (84.36%, 69.06%), Group E (35.04%, 28.09%), Group F (16.17%, 10.36%), and Group G (66.84%, 58.02%). Treatment with CO showed significant ($P < 0.01$) enhancement in the weight of prostate and seminal vesicle in Group C (36.38%, 27.74%) and in Group D (84.63%, 41.47%) compared to Group A. However, significant ($P < 0.01$) rises in the weight of prostate and seminal vesicle were noted in the Group E (315.5%, 132.43%), Group F (434.48%, 189.72%) and in Group G (112.06%, 35.67%) as compared to Group B [Table 2].

The weight of epididymis was decreased significantly ($P < 0.01$) in Group B (63.46%), Group E (20.89%), Group G (49.53%) and non-significantly ($P > 0.05$) in Group F (5.10%) compared to Group A. On the other hand, significant ($P < 0.01$) increase in epididymis weight was observed in

Group E (116.52%), Group F (159.74%) and in Group G (38.13%) compared to DLM exposed Group [Table 2].

Oxidative stress parameters

Table 3 showed significant ($P < 0.01$) increase in LPO in Group B, E (80.42%, 20.01%) and non-significant ($P > 0.05$) in Group F and G (5.48%, 55.21%). However, LPO level significantly ($P < 0.05$) decreased in Group C (22.00%) and Group D (26.04%, $P < 0.01$) in comparison to Group A. CO treatment significantly ($P < 0.01$) reduced LPO level in Group E, F and G (30.15%, 41.53%, 13.97%), respectively, as compared to Group B. It was observed that GSH level significantly ($P < 0.01$) decreased in Group B, E and G (65.79%, 30.78%, 70.13%), respectively, and non-significantly ($P > 0.05$) in Group F (4.98%) as compared to Group A. It was observed that GSH level increased significantly ($P < 0.01$) in Group C, D, E and F (37.94%, 41.47%, 102.07% and 177.69%, respectively) and non-significantly ($P > 0.05$) decreased in Group G (12.65%) as compared to Group B. Significantly

($P < 0.01$) decrease in activities of CAT, SOD, GST and GPx (36.63%, 41.74%, 20.65%, 61.14%, respectively) in Group B, (24.37%, 21.84%, 39.11%, 31.47%, respectively) in Group E, (17.71%, 16.42%, 6.32%, 20.70%, respectively) in Group F and (37.56%, 45.42%, 21.88%, 62.52%, respectively) in Group G was observed. Significant ($P < 0.05$) increase in activities of these enzymes was observed in Group C (7.00%, $P < 0.01$ - 8.94%, 136%, 32.53%, respectively) and D (10.29%, 15.32%, 230%, 40.96%, respectively) when compared with the control Group A. However, significant ($P < 0.01$) increase in CAT, SOD, GST and GPx activities was observed in Group E (19.41%, 34.15%, 12.61%, 76.34%) and Group F (29.92%, 43.96%, 18.06%, 104.06%) as compared with Group B. Significant ($P < 0.01$) decrease in CAT, GST (1.43%, 1.54%) and non-significant ($P < 0.05$) decrease in SOD, GPx (6.31%, 45.42%) was reported in Group G as compared to Group B. GR level increased significantly ($P < 0.01$) increased in Group C (135.51%) and D (229.79%) and non-significantly ($P > 0.05$) in Group G (2.87%) when compared with Group A. However, significant ($P < 0.01$) decrease in GR level was reported in Group B (64.70%), E

Table 1: Effect of CO and DLM on body weight of the rats

Parameter	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Day 1 body weight (g)	250.03±1.50	251.16±2.08	249.33±2.02	250.83±3.00	254.5±2.09	251.33±1.92	252.83±2.35
Day 60 body weight (g)	253.05±2.14	210.83±0.83**	257.66±3.72	262.16±2.08**	244±1.15**	245.5±0.76	226.6±1.15**

Mean±SEM (n=6 animals/group). *P>0.05, **P<0.01 compared to group A control. Group A- Control; Group B- DLM; Group C- Treatment 1; Group D- Treatment 2; Group E- Combination 1; Group F- Combination 2; Group G- Metabolized.

Table 2: Effect of CO and DLM on reproductive organs weight

Parameter	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Testis (g)	1.49±0.02	1.09±0.02**	1.55±0.05	1.60±0.03	1.41±0.04 ^c	1.51±0.03 ^c	1.24±0.08 ^{**a}
Prostate (g)	0.37±0.01	0.05±0.01**	0.50±0.01**	0.68±0.01**	0.24±0.01 ^{**c}	0.31±0.00 ^{**c}	0.12±0.00 ^{**c}
Seminal vesicle (g)	0.59±0.00	0.18±0.00**	0.74±0.01**	0.84±0.01**	0.43±0.1 ^{**c}	0.53±0.01 ^{**c}	0.25±0.01 ^{**c}
Cauda epididymis (g)	0.64±0.1	0.23±0.01**	0.73±0.01**	0.80±0.01**	0.51±0.0 ^{**c}	0.61±0.00 ^c	0.32±0.01 ^{**c}

Mean±SEM (n=6 animals/group). *P>0.05, **P<0.01 compared to group A control, a= P > 0.05, c= P < 0.01 compared to DLM group.

Table 3: Effect of CO and DLM on testicular oxidative stress parameters Wistar rats

Parameter	Group A	Group B	Group C	Group D	Group E	Group F	Group G
LPO (nmoleMDA/h/g tissue)	3.463±0.12	6.24±0.13**	2.86±0.14*	2.56±0.13**	4.36±0.11 ^{**c}	3.65±0.12 ^c	5.37±0.13 ^c
GSH (µmole/g tissue)	2.48±0.15	0.85±0.02**	3.43±0.16**	3.52±0.17**	1.72±0.02 ^{**c}	2.36±0.08 ^c	0.74±0.01 ^{**a}
CAT (µmole/min/mg)	68.03±0.23	43.11±0.17**	72.83±0.33**	75.07±0.33**	57.48±0.21 ^{**c}	56.01±0.25 ^{**c}	42.49±0.20 ^{**c}
SOD (µmole/min/mg protein)	33.42±0.14	19.47±0.13**	36.41±0.18*	38.54±0.18**	26.12±0.19 ^{**c}	28.03±0.26 ^{**c}	18.24±0.30 ^{**a}
GST (µmole/min/mg protein)	87.43±0.16	69.37±0.16**	92.05±0.25**	94.10±0.05**	78.13±0.20 ^{**c}	81.90±0.30 ^{**c}	68.30±0.32 ^{**c}
GPx (nmole/min/mg protein)	6.13±0.06	2.38±0.15**	8.13±0.27**	8.64±0.35**	4.20±0.29 ^{**c}	4.86±0.28 ^{**c}	2.29±0.14 ^{**a}
GR (nmole/min/mg protein)	2.25±0.13	0.79±0.19**	5.31±0.16**	7.44±0.14**	1.37±0.14 ^{**c}	2.23±0.11 ^c	2.32±0.14 ^c

Mean±SEM (n=6 animals/group). *P>0.05, *P<0.05, **P<0.01 compared to Group A control, ^aP>0.05, ^cP<0.01 compared to DLM group. Group A: Control; Group B: DLM; Group C: Treatment 1; Group D: Treatment 2; Group E: Combination 1; Group F: Combination 2; Group G: Metabolized. LPO: Lipid peroxidation, GSH: Glutathione, CAT: Catalase, SOD: Superoxide dismutase, GST: Glutathione-S-transferase, GPx: Glutathione peroxidase, GR: Glutathione reductase

(39.06%), and non-significant ($P > 0.05$) in Group F (1.01%) as compared to Group A. Results showed that GR level significantly ($P < 0.01$) increased in Group C (567.25%), D (834.62%), E (72.64%), F (180.42%), and G (191.46%) when compared with Group B.

Sperm characteristics

Sperm head count and sperm motility were significantly ($P < 0.01$) decreased in Group B (47.88%, 78.84%), Group E (22.64%, 23.43%), Group F (11.03%, 10.64%) and Group G (34.33%, 61.47%), respectively, whereas significant ($P < 0.01$) increases was observed in Group C (10.46%, 8.46%) and Group D (28.77%, 13.69%), respectively, as compare to Group A. In combination and metabolized groups significant ($P < 0.01$) increase in sperm head count and sperm motility was reported in Group E (48.13%, 225.25%), Group F (70.36%, 314.57%) and Group G (25.75%, 78.54%), respectively, when compared with DLM treated Group B. Sperm abnormality was significantly ($P < 0.01$) increased in Group B (713%), Group E (129.52%), Group F (73.16%) and Group G (426.83%) as compared to Group A. Treatment with CO reduced sperm abnormality non-significantly ($P > 0.05$) in Group C (13.33%,) and significantly ($P < 0.05$) in Group D (21.64%,) when compared with Group A and significantly ($P < 0.01$) in Group E (71.37%), Group F (78.72%) and Group G (35.27%) as compared to Group B [Table 4].

Reproductive hormones

Significant ($P < 0.01$) decrease in T, FSH and LH level was observed in Group B (58.13%, 74.60%, 70.09%), Group E

(20.08%, 25.57%, 25.05%), Group F (2.57%, 4.19%, 7.54%) and Group G (45.34%, 50.31%, 56.92%), respectively, as compared to control Group A. Significant ($P < 0.01$) increase in T, FSH and LH was reported in Group C (14.77%, 12.81%, 12.26%) and Group D (31.72%, 26.75%, 23.63%), respectively, as compared to Group A. In comparison to Group B, all three hormonal levels were significantly ($P < 0.01$) enhanced in Group E (90.89%, 193.09%, 146.86%), Group F (132.74%, 277.30%, 204.54%) and Group G (30.15%, 95.66%, 41.86%) [Table 5].

Steroidogenic enzyme

The activity of 3β HSD and 17β HSD was significantly ($P < 0.01$) decreases in Group B (92.35%, 85.15%), Group E (31.42%, 24.70%), Group F (10.41%, 6.26%) and Group G (71.89%, 69.09%), respectively, in comparison to control Group A. It was observed that the activity of both steroidogenic enzymes was significantly ($P < 0.01$) increases in Group C (13.11%, 16.14%), Group D (35.21%, 27.10%) as compared to Group A. In comparison to DLM treated group, both the enzymes were significantly ($P < 0.01$) increases in Group E (796.74%, 407.08%), Group F (1071%, 531.30%) and Group G (267.47%, 108.40%), respectively [Table 6].

Histological effects

In the control group, testicular tissue showed normal arrangement of basement membrane with well-organized leydig cells (OLCs) and sertoli cell (CS), lumen filled with spermatids (LST), spermatozoa with tail (SZT) and well-developed spermatogonia (WSG) [Figure 1a]. DLM treated

Table 4: Effect of CO and DLM on reproductive parameters on Wistar rats

Parameter	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Sperm count $\times 10^4$	449.01 \pm 0.44	234.47 \pm 0.91**	496.24 \pm 0.47**	542.27 \pm 0.65**	347.33 \pm 0.46** ^c	399.45 \pm 0.61** ^c	294.86 \pm 0.38** ^c
Sperm motility (%)	72.89 \pm 0.4	15.71 \pm 0.33**	79.06 \pm 0.55**	82.87 \pm 0.34**	55.81 \pm 0.23** ^c	65.13 \pm 0.50** ^c	28.05 \pm 0.37** ^c
Sperm abnormality (%)	11.55 \pm 0.24	94.01 \pm 1.11**	10.01 \pm 0.33*	9.05 \pm 0.36*	26.91 \pm 0.55** ^c	20.00 \pm 0.40** ^c	60.85 \pm 0.47** ^c

Mean \pm SEM ($n=6$ animals/group). * $P > 0.05$, ** $P < 0.05$, *** $P < 0.01$ compared to control. ^c $P < 0.01$ compared to DLM. Group A: Control; Group B: DLM; Group C: Treatment 1; Group D: Treatment 2; Group E: Combination 1; Group F: Combination 2; Group G: Metabolized. CO: *Curculigo orchioides*, DLM: Deltamethrin, SEM: Standard error of mean

Table 5: Effect of CO against DLM induced reproductive alteration in hormone levels

Parameter	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Testosterone (ng/ml)	3.38 \pm 0.09	1.41 \pm 0.06**	3.88 \pm 0.07**	4.45 \pm 0.09**	2.70 \pm 0.03** ^c	3.29 \pm 0.04* ^c	1.85 \pm 0.03** ^c
FSH ((ng/ml)	4.26 \pm 0.01	1.08 \pm 0.00**	4.81 \pm 0.01**	5.41 \pm 0.02**	3.17 \pm 0.01** ^c	4.09 \pm 0.00** ^c	2.12 \pm 0.00** ^c
LH (ng/ml)	5.14 \pm 0.02	1.56 \pm 0.01**	5.71 \pm 0.01**	6.36 \pm 0.02**	3.85 \pm 0.01** ^c	4.75 \pm 0.03** ^c	2.21 \pm 0.02** ^c

Mean \pm SEM ($n=6$ animals/group). * $P > 0.05$, ** $P < 0.01$ compared to control. ^c $P < 0.01$ compared to DLM. Group A: Control; Group B: DLM; Group C: Treatment 1; Group D: Treatment 2; Group E: Combination 1; Group F: Combination 2; Group G: Metabolized. CO: *Curculigo orchioides*, DLM: Deltamethrin, SEM: Standard error of mean, FSH: Follicles stimulating hormone, LH: Luteinizing hormone

group showed thin basement membrane (TBM), necrosis of sertoli cells (NCS), vacuolation of lumen (VLM), necrosis of leydig cells (NLCs), and arrested stages of spermatogenesis (AS) including disorganized spermatogonia (DSG) [Figure 1b]. Rats treated with CO at 100 mg/kg bw showing thick basement (TBM), increased spermatogonia (IG), well-developed leydig cells (WLC), increased spermatogenesis, organized cell of sertoli (OCS), and lumen (DLM) filled dense fluids [Figure 1c]. Treatment with CO at 200 mg/kg bw showing histological change including bulky basement (BBM), crowded structure of spermatogonia (CSG), densely packed primary spermatocytes (PSC), secondary spermatocyte (SSC), lumen filled with spermatids (LMS), OLCs, and well-shaped cell of sertoli (WCS) [Figure 1d]. Photograph of group received combination of DLM and plant extract at 100 mg/kg body weight showing improved basement membrane (IBM), less necrosis of leydig cell (LLC), size of vacuole of the lumen decreased (VLM), number of spermatids were

recovered (ST), and shape of cell of sertoli less disorganized (SCS) [Figure 1e]. Group treated with combination of DLM and plant extract at 200 mg/kg body weight showing less disorientation of basement membrane (LBM), cell of sertoli least affected (LCS), no restriction of spermatogenesis is observed including unaffected primary spermatocyte (UPS), secondary spermatocyte (USS) and compact structure of spermatogonia is observed (SGC) [Figure 1f]. In metabolized group, basement membrane was porous (PBM), spermatogonia were damaged (SGD), PSC, SSC were observed but lumen filled with dead spermatozoa (LMD) and cell of sertoli (CSD) were disorganized [Figure 1g].

DISCUSSION

DLM exposure resulted in decrease in body and sex organs weight in this investigation. It has been reported earlier that

Table 6: Effect of CO and DLM on steroidogenic enzyme (3β HSD and 17β HSD)

Parameter	Group A	Group B	Group C	Group D	Group E	Group F	Group G
3β HSD (pg/ml)	3.21±0.06	0.24±0.00**	3.69±0.08**	4.37±0.07**	2.20±0.02**c	2.88±0.02**c	0.90±0.03**c
17β HSD (ng/ml)	4.08±0.01	0.60±0.00**	4.74±0.05**	5.19±0.03**	3.06±0.02**c	3.83±0.03**c	1.26±0.01**c

Mean±SEM (n=6 animals/group). *P>0.05, **P<0.01 compared to control. °P<0.01 compared to DLM. Group A: Control; Group B: DLM; Group C: Treatment 1; Group D: Treatment 2; Group E: Combination 1; Group F: Combination 2; Group G: Metabolized. CO: *Curculigo orchioides*, DLM: Deltamethrin, SEM: Standard error of mean, HSD: Hydroxysteroid dehydrogenase

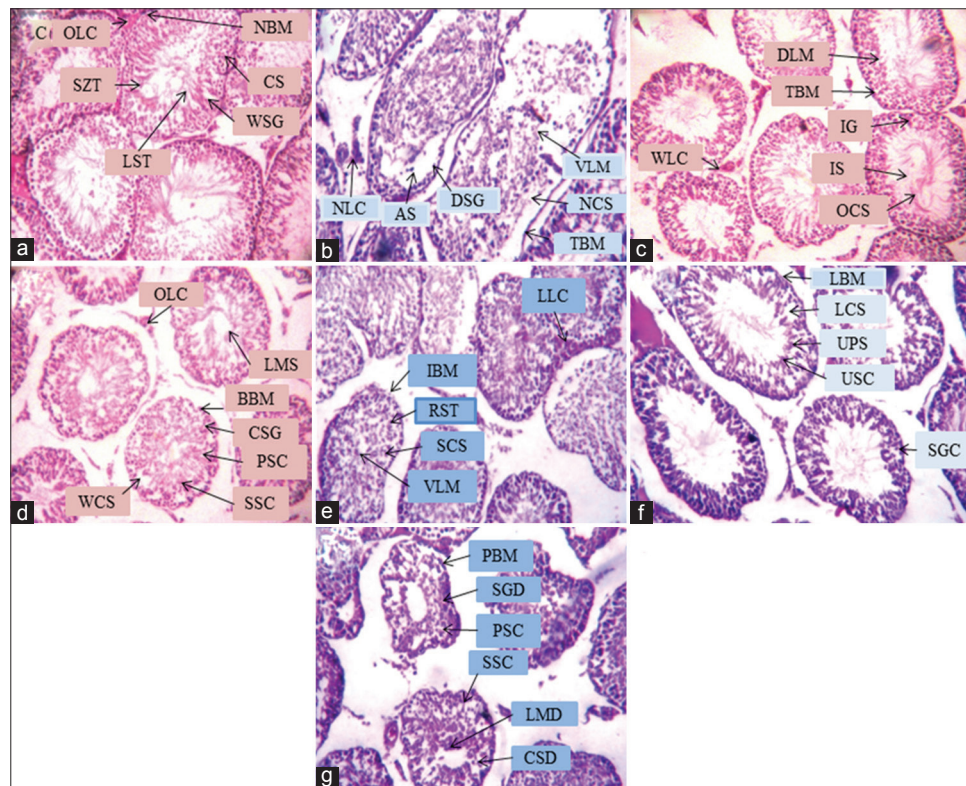


Figure 1: Photomicrograph of rat testes stained with hematoxylin and eosin: (a) Normal testes *ad libitum* diet and water for 60 days, (b) deltamethrin (DLM) 2 mg/kg bw for 60 days, (c) *Curculigo orchioides* (CO) (100 mg/kg bw) for 60 days, (d) CO (200 mg/kg bw) for 60 days, (e) DLM 2 mg/kg bw along with CO (100 mg/kg bw) for 60 days, (f) DLM 2 mg/kg bw along with CO (200 mg/kg bw) for 60 days, (g) DLM 2 mg/kg bw will be allowed to metabolized for 1 month after 60 days exposure

the number of mature spermatogenic cells contributes toward the weight of testis.^[30] Reduction in weight of the testis may be due to decrease in number of spermatogenic cells, inhibition of spermatogenesis and steroidogenic enzyme activity. Kim *et al.*^[31] reported that exposure to synthetic pyrethroids led to significant reduction in weight of the androgen-dependent accessory sex tissue and sex organs. It is reported that decrease in T, FSH and LH decreases weight of sex organs.^[32] DLM exposure resulted in decrease in T, FSH, and LH in this study. Decrease in weight of testis, prostate, seminal vesicle and epididymis may be either due to decrease in concentration of T, FSH, and LH or increase in the formation of reactive oxygen species.

DLM is lipophilic in nature, it may easily cross the blood testes barrier, accumulates in testicular cells and induces free radicals generation. Increase in LPO in this study on DLM exposure may be either due to increase in free radicals or membrane damage. There are reports of increase in level of LPO on dimethoate and DLM exposure.^[10] GSH level was significantly decreased on DLM exposure. The decrease in GSH level may be due to imbalance in pro-oxidant/antioxidant system. GSH used to maintain the thiol-disulfide status of the cells.^[33]

Exposure of DLM in this study also reduced the activities of CAT and SOD, possibly due to generation of reactive oxygen species and oxidative damage SOD in testes since these two antioxidant enzymes are interlinked.^[34] Our finding showed that level of GST, GPx and GR were decreased on DLM exposure. The decrease in activities of these enzymes may be due to are dependent on GSH. Reduced level of these enzymes due to accumulation of DLM causes increased LPO and oxidative stress and consequently lead to testicular toxicity. Earlier decrease in non-enzymatic and enzymatic antioxidants have been reported by Sharma *et al.*^[35] in cypermethrin treated rats.

The sperm count and motility decreased in DLM treated group, possibly due to oxidative stress causing germ cell apoptosis. ROS initiate a chain of reactions that ultimately leads to apoptosis. The process of apoptosis may also be accelerated by ROS-induced DNA damage and ultimately decrease the sperm count and motility.^[36] Sperm abnormality increases in DLM exposed group which may be due to the injury and destruction of germ cells as well as decrease in reproductive hormones.

The level of T, FSH and LH were significantly decreased in DLM treated group, may be due to increased apoptosis and decreased proliferation of testicular cells, detachment of germ cells from the epithelium due to increased ROS. It may also be possible that toxic effects of DLM may result in the failure of the pituitary to secrete FSH and LH, resulting in testicular dysfunction. Ismail and Mohamed^[37] reported DLM induced testicular injury in rats. Steroidogenesis is mainly regulated by pituitary gonadotropins such as LH and FSH hormones.

Exposure to environmental contaminants has adverse effects on testicular function by decreasing pituitary LH secretion and reducing leydig cell steroidogenesis.^[9] Qu *et al.*^[38] reported that fenvalerate, a pyrethroid insecticide inhibited progesterone production by attenuating cAMP generation and inhibiting cytochrome P450 side chain cleavage complex (P450scc).

DLM exposure significantly decreased the activities of testicular (3 β HSD) and (17 β HSD) in testes. It may be possible that DLM inhibited progesterone production by attenuating cAMP generation and inhibiting cytochrome P450 side chain cleavage complex (P450scc) which catalyzes synthesis of steroidogenic enzymes as well as T.

Histological examination of testis of DLM exposed rats revealed many alterations such as thin basement membrane, necrosis of sertoli cells, vacuolation of lumen, necrosis of leydig cells, and arrested stages of spermatogenesis including disorganized spermatogonia. Morphological alterations of testes architecture may be due to induction of oxidative stress induced by DLM. Sakr and Al-Amoud^[39] observed irregular seminiferous tubules with reduced spermatogenic cells and damaged interstitial tissue after exposing rats to DLM.

Administration of CO increased the body weight as well as weight of testis and accessory sexual organs. Increased weights body weight may be due to radical scavenging activity of hydroalcoholic extract of CO and increase in protein mass. CO has been reported to contain steroidal phytochemicals.^[40] The presence of steroidal phytochemicals may enhance the biosynthesis of reproductive hormones, maintaining spermatogenic index and increasing the weight of testis and other accessory sex organs, in this study. The increase in body weight as well as weight of testis and accessory sex organs may also be due to the anabolic activities of the steroidal phytochemicals. Treatment of CO showed decrease in LPO and increase in GSH, CAT, SOD, GST, GPx, and GR. The study showed that CO possesses good antioxidant and ROS scavenging properties. Antioxidant property of hydroalcoholic extract of CO has been previously reported by Onkar *et al.*^[41] Owing to its antioxidant properties, CO improved the antioxidant defense, reduced DLM induced cell damage and enhanced activities of vital antioxidant enzymes. CO treatment 2 at 200 mg/kg bw in Group D was more effective as compared to treatment 1 at 100 mg/kg bw, indicating the effect was dose dependent.

Treatment with CO reversed DLM induced loss of sperm count and motility. The sperm abnormalities were also reduced significantly. Treatment with CO increased T-level. The increase in T-level may either due to direct protective role of CO against DLM induced oxidative stress in germ cells and sertoli cells or due to increase in mass of sex organs. CO also increased the concentration of secretion of FSH and LH, possibly via stimulating secretion of gonadotropin releasing hormone. CO treatment also increased the level of testicular

3 β HSD and 17 β HSD further enhancing the biosynthesis of testosterone. Increase in T, FSH and LH level positively regulated the process of spermatogenesis, enhancing sperm counts as well as increasing the number of functional and motile sperms. Certain phytochemicals from medicinal plants have previously been reported to increase sperm counts^[42] as well as exerted aphrodisiac effect.^[43] CO treatment also normalized the histological disturbances induced by DLM exposure. This ameliorating effect was dose dependent.

The findings of this study indicated that hydroalcoholic extract of CO rhizome possess reductive system enhancement and fertility improvement capabilities. The extract may be further explored for isolation and purification lead compounds and safety studies.

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