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Evaluation of ethephon induced oxidative stress to gonadal disorder and its amelioration by ethanolic extract of shoot of *Bambusa balcooa* Roxb. in albino rat

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Ethephon (ETP) is an organophosphorous pesticide widely used in agriculture as a plant growth regulator as it promotes ripening and maturation of fruits and vegetables, respectively. People are exposed to ETP via consumption of artificially ripened and marketed fruits and vegetables which is of great concern to public health hazard. The current study aimed to evaluate the protective effect of *Bambusa balcooa* shoot extract against ethephon induced oxidative stress and gonadal function in albino rats. A total of 60 healthy Swiss Albino rats consisting of 35 males and 25 females were divided into five groups. The rats were subchronically exposed to two doses of ETP, *viz*. 15mg/KG bw and 30 mg/KG bw. ETP toxication significantly decreased weight of the testes and accessory sex organs such as epididymes and vas deferens. It significantly decreased sperm count, sperm motility and sperm viability but significantly increased sperm morphological abnormalities. In female rat, exposure of ETP gradually decreased the duration of proestrus, estrous and metestrous phases of estrous and concomitantly increased the duration of diestrus phase. Administration of *Bambusa balcooa* shoot extract ameliorated these alterations caused by the toxic impacts induced by ETP in albino rats. So, it could be concluded that *Bambusa balcooa* shoot extract can be considered as a preventive and curative natural substance against ethephon induced oxidative stress and gonadal function in albino rat.

Key words: Artificial ripening agent, Ethrel, Gonadal function, Oxidative enzyme, Reproductive hormone

The demand for fruits and vegetables are increasing daily as they contain different types of vitamins and minerals and have antioxidant property to fight against various health problems. The supply of fruits and vegetables is not enough to meet consumers' demands as natural ripening and maturation process is slow, uneven and unpredictable. Therefore, to provide regular supply of fruits and vegetables as well as to earn a quick profit, the retailers and traders use various synthetic chemicals such as Calcium carbide, Ethephon, Ethylene, Acetylene gas, Carbon monoxide, Potassium sulphate, *etc*. to fasten the ripening and maturation processes of fruits and vegetables[1]. Use of these chemicals make the fruits and vegetables more appealing to consumers with uniformly bright coloured and hence results in better marketing[2]. These chemicals are also known to result in poor flavour and aroma as well as reduce nutritional value and can possibly be toxic to human beings[3]. Amongst such chemicals, ethephon (ETP) or 2-chloroethylphosphoric acid, a plant growth regulator is extensively used to promote pre- and post-harvest ripening of fruits and vegetables. ETP is mostly used because fruits when treated with it become softer, sweeter, and look more appealing[4].

ETP easily penetrates tissues and decomposes to ethylene, which is the active metabolite. Further conversion produces ethylene oxide, a carcinogen. Studies also reported toxic effects of ETP. Haux *et al*. reported that ETP exposure can inhibit the activity of plasma cholinesterase (ChE) in humans, dogs, rats and mice[5]. ETP has also been found to reduce DNA and RNA concentrations in different tissues, viz., brain, liver, and kidney[6]. Therefore, use of this chemical for ripening and maturing of fruits and vegetables poses a risk to human health. Given that it is not possible for us to completely avoid such artificially ripened foods and vegetables, thus attempt can be made to mitigate the toxic effects through food itself such as medicinal plants and herbs. Northeast region of India is rich in floral diversity, many of them with medicinal importance. A number of ethnic tribes living in this zone have the traditional knowledge of using different plants as remedies for various health ailments. Among such indigenously used plants is the bamboo plant, whose shoots form one of the essential ingredients in dishes prepared by tribal communities of NE region. It

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is consumed either in fresh form during harvesting season or dried, fermented or pickled forms during offseason[7]. Bamboo shoots are rich in protein, carbohydrates, and minerals such as K, Ca, Mn, Zn, Cu and are good source of vitamin A, B6 and E[8]. Bamboo shoots are widely used by the people of NE region to improve appetite and digestion, control blood pressure, treat kidney diseases, general weakness and other cardiovascular diseases. It is also used to treat various reproductive health related problems such as irregular menstrual cycle, reduce labor pain and heavy bleeding during postpartum, *etc*,[9].

People are exposed to ETP *via* consumption of artificially ripened and marketed fruits and vegetables which is of great concern to public health. Also the reproductive toxicity on gametogenic activity of ETP has not been thoroughly studied. Therefore, owing to its antithyroidal, antioxidant and scavenging activity[10] and its uses in various health related problems, bamboo shoot may play a role to mitigate the chemical induced toxic or oxidative stress. On this contemplated background, this study was carried out to evaluate the effect of ETP induced oxidative stress on gonadal function and its ameliorative activity by ethanol extract of shoot of *Bambusa balcooa* selecting albino rat as experimental model.

Materials and Methods

Experimental animals

In the main experimental set up 65 male and 25 female Swiss albino rats of Sprague Dawley strain were selected for the study. The animals were procured from Department of Zoology, Gauhati University. Sexually matured male and female albino rats weighing between 130g and140g and were housed in separate propylene cages bedded with paddy husk and the animals were fed with standard rodent food and water *ad libitum* throughout the period of study. The animals were kept under uniform laboratory conditions of light (12 h light/dark cycle), room temperature (25±2ºC) and relative humidity (52±5%). The animals were acclimatised to the laboratory conditions 15 days prior to the commencement of the experiment. Experiment was carried out according to the guidelines of CPCSEA and after approval of Institutional Animal Ethical Committee vide certificate of approval 8/IAEC/CU/05/01/2021.

Test substance and Reagents

The test substance etephon (ETP) was obtained from Loba Chemie, Mumbai. Ethanol was purchased from Hi media. Chloroform, formaldehyde, glacial acetic acid and diethyl ether were purchased from Merck (Germany). Haematoxylin and Eosin were purchased from Sigma-Aldrich.

Experimental Design

In the experimental setup, animals were divided into five groups, Group I- Control: water *ad libitum;* Group II- Treated: ETP at a dose of 15mg/kg body wt./mL/day for 90 days; Group III- Treated: ETP at a dose of 30 mg/kg body wt./mL/day for 90 days; Group IV- BS extract group:100mg/kg body wt. BS (Bamboo shoot) extract after withdrawal of ETP treatment 15mg/kg body weight; Group V-BS extract group:100mg/kg body wt. BS (Bamboo shoot) extract after withdrawal of ETP treatment 30 mg/kg body weight. In each group 5 male rats were put for study of male gonadal disorder with observations conducted at every 30 days over a period of 90 days. Similarly, 5 number of female rats were put in each group for study of estrous cycle as well as related female gonadal disorder studies for a period of 90 days.

The treated groups were fed with ETP at a dose of 15mg/kg and 30mg/kg body weight daily for a period of 90 days during morning hours (8.20 AM to 9.30 AM). The rats which received same volume of water (1mL/kg body weight/day) served as control rats. During the experiment, the body weight of the rats was recorded in both control and treated groups at every three days intervals and behavioural changes were observed. At every 30 days interval, male rats were sacrificed under mild etherisation (diethyl ether) for analysis of male reproductive parameters such as testicular and accessory sex organs weight, spermatological parameters like sperm count, sperm motility and viability as well as sperm cell morphology and fertility test. Prior to sacrifice body weight of the animals were recorded and later the weight of sex organs such as testis, epididymes and vasa-differentia were noted *via* dissection for analyzing the relative proportion of organ weight with respect to body weight. To ascertain the possible effect of the chemical on estrous cycle, experiment was also conducted on female rats for the same duration.

Estrous cycle

The estrous cycles were monitored by a method described by Marcondes, *et al.*[11] vaginal fluid was collected with a glass pipette filled with normal saline (NaCl 0.9%). The fluid was then placed on a clean

glass slide and a thin smear was prepared. The slide was then stained with Giemsa (pH 6.8) and cell types and the morphological abnormalities of three distinct cell types *viz*., epithelial, cornified and leucocytic cells were recorded under microscopic observation (Olympus) at 400× magnification. Duration and proportions among all the cell types of different phases of estrous cycle were determined according to Long & Evans[12].

Semen collection

The epididymis along with the testis were removed and weight was recorded immediately. For the study of spermatological parameters, the caudal epididymes was separated quickly from the testis and gently teased to collect the semen on a slide.

Sperm motility and viability

Sperm collected from teasing of known weight of cauda epididymes in specific volume of physiological saline (0.9% NaCl) was immediately taken on a pre warmed clean slide. The sperm suspension was then observed within 5 min and scored under light microscope using magnification 400× according to standard method of Morrissey *et al*. Number of both motile and non motile sperm were counted in at least ten separate randomly selected fields and motility was expressed in percentage.

The viability of the sperm *i.e*. percentage of spermatozoa was studied using eosin stain. The teased semen was taken on a slide and 2-4 drops of stain were applied. The unstained sperms recognized as live or motile, whereas stained sperms were found to be non-motile or dead. Both stained and unstained sperm cells were counted using 400× magnification and percentage of viability was calculated from the average recorded value of each.

Sperm count

The excised epididymes was gently teased in 10mL of diluting fluid (5% Sodium bicarbonate with addition of 1mL of 40% formaldehyde) to release the spermatozoa from the tubules. The sperm count was done using counting chamber of improved Neubaur haemocytometer according to the routine method of Freund & Carol[13].

Sperm morphology

The sperm morphology was studied by making sperm smear on the slide and left for air dry. The slides were then stained with eosin stain for 5-7 min and observed under microscope with an oil immersion with 100× objective. The morphological deformities of sperm cells were recorded and their percentage was calculated using the method described by Wyrobek & Bruce[14].

Fertility test

A separate group of 45 fertile female rats were maintained for fertility test. A group consisting both male (n=15) and fertile proestrus female rats (n=45) ranging between 120 and 130 gm body weight were selected for fertility test. Three male rats from each treated group for 60 days of treatment were considered for performing mating exposure test on account of possible progressive effect of the chemical. All proestrus females (control) cohabitated with treated males in a ratio of 3:1, whose cohabitation commenced on 1st day and remain for 5 days according to Dhawan & Sharma[15]. A similar control group was maintained for fertility test in which fertile proestrus females were cohabitated with control males in a similar manner. The vaginal plug and presence of sperm in the vaginal smear was checked for possible mating. Females were separated and resultant pregnancies were noted. When the pregnant females gave birth, the number of litters delivered and their weights were recorded.

A fertility test was calculated using the following formula given by Raji *et al*.[16]

% Fertility= (Pregnant female)/(Mated female) × 100

Bamboo shoot extract preparation

Bamboo shoots were washed with distilled water, cut into thin slices and then shade dried for 7-10 days in 25-27°C (day time). Dried bamboo shoots were powdered in a stainless grinder machine and then submitted in Soxhlet apparatus (boiling point: 60-80˚C for 8-10 h). Now the extracted solvent was filtered through Whatman no.1 filter paper (0.2µ). The filtered extract solvent was concentrated at reduced pressure (at 45°C) were diluted in methanol which was sterilized by filtration.

Histological studies

Histopathological studies of the reproductive organs of both the male and female rats were performed. After sacrifice, the ovary and testis were removed and fixed in Carnoy's fixative overnight. The tissues were dehydrated using different alcohol grades and mounted in paraffin blocks. The sections of tissue were cut in 4 microns using rotary microtome. The tissues were then stained with H & E

stain by method of Luna, (1968)[17] and observed under microscope.

Biochemical estimation of antioxidant enzyme profiles

The estimation of levels of activity of superoxide dismutase (SOD) and catalase (CAT) were carried out by preparing respective tissue extracts from testis after animal sacrifice. Lipid peroxidation activity at tissue level was also determined by estimating the amount of malondialdehyde (MDA) generation. Levels of SOD activity was assayed by using SOD assay kit (Abcam Ltd, No. ab65354). The absorbance was measured at 543 nm and results were expressed in units/mg protein, where one unit of enzyme activity was defined as the amount of superoxide dismutase capable of inhibiting nitrite formation upto 50% under assay conditions. Catalase activity was estimated by using Catalase assay kit (Cayman chemicals, No. 707002) obtaining colorimetric method, where OD was measured at 610 nm. The Catalase activity was expressed in IU/mg protein. Again, MDA levels in tissue extracts were assayed by MDA assay kit (Abcam Ltd., no. 118970) and colour reaction measured at 540 nm. The MDA levels were expressed as n-mole/mg protein. For calculation of GPx activity at tissue level, the method of Rotruck *et al*.[18] was followed. The GPx activity was expressed as units/mg protein.

Statistical analysis

The data recorded from the above experimental work were subjected to statistical analysis which were presented as mean ± SE. The differences among different groups were analyzed using One Way Analysis of Variance (ANOVA) followed by Duncan's multiple test for comparison. The statistical significance for comparison of differences among the different groups were tested by *t-test*. The significant level was considered at $P<0.05$ and $P<0.01$ of probability level. For sperm abnormalities, the data was presented as percentage form and their significant level were analyzed using *t-test* and *chi-square* test. Graphical representation of data has been carried out on Microsoft Excel 2007.

Results

Estrous cycle

A regular estrous cycle was exhibited by the control rats showing normal duration of each phase and number (ranged from 5.93±0.35 to 6.12±0.05) of the estrous cycle. Whereas a significant ($P<0.05$) decrease in number of estrous cycle and duration of different estrous phases were recorded in both the treated groups. Further, depletion of duration of estrous phases especially proestrus, estrous and metestrus along with concomitant significant increase of duration of diestrus phases with elevated diestrus index were also noted in both the treated groups i.e. group II and group III. However, variation in estrous cycle was highly significant ($P\leq0.05$) in 30 mg ETP treated group as compared to 15 mg treated group as well as the control. In group IV and V, the number of estrous cycles were found to be similar with the control group. There was also no significant difference between the duration of each estrous phase and diestrus index exhibited by the animals of group IV and V with that of control group. The details of these variation in estrous cycle are shown in Table 1.

Table 1 — Impact of BS in ETP induced effect on number of estrous cycle and duration of different estrous phases (values are mean ± SE of 5 animals)

Treatment days	Groups	Dose (mg/kg body wt.)	Number of cycles	Duration in days (M±SE)				Diestrus index
				Proestrus	Estrous	Metestrus	Diestrus	
30 days	I	Control	5.93^{a}±0.35	4.91^{a}± 0.36	7.75^{a}±0.24	5.49^{a}±0.52	11.83^{a}±0.31	39.43^{a}
	II	15	4.45^{b}±0.71	3.95^{b}± 0.22	6.67^{b}±0.20	4.93^{b}±0.43	14.45^{b}±0.37	48.17^{b}
	III	30	4.32^{b}± 0.16	3.02^{b}± 0.27	6.07^{b}±0.31	4.58^{b}±0.39	16.31^{c}±0.21	54.36^{c}
60 days	I	Control	6.03^{a}±0.35	4.78^{a}±0.24	7.85^{a}±0.19	5.61^{a}±0.15	12.76^{a}±0.23	42.53^{a}
	II	15	3.93^{b}±0.43	2.93^{c}±0.55	5.71^{c}±0.36	3.65^{c}±0.32	17.71^{c}±0.41	59.03^{c}
	III	30	3.61^{c}±0.26	2.47^{c}±0.33	5.39^{c}±0.18	3.41^{c}±0.27	18.73^{c}±0.23	62.43^{c}
90 days	I	Control	6.12^{a}±0.05	5.01^{a}±0.12	7.91^{a}±0.21	5.42^{a}±0.19	11.65^{a}±0.15	38.83^{a}
	II	15	3.24^{c}±0.51	2.65^{c}±0.45	4.91^{c}±0.37	3.01^{c}±0.59	19.43^{c}±0.42	64.77^{b}
	III	30	2.86^{c}±0.41	2.01^{c}±0.31	4.09^{c}±0.41	3.04^{c}±0.65	20.86^{c}±0.58	69.53^{b}
90 days	IV	100 mg BS after withdrawal of 15mg ETP	6.17^{a}±0.16	4.87^{a}±0.12	6.55^{a}±0.39	5.77^{a}±0.27	11.35^{a}±0.43	37.92^{a}
	V	100 mg BS after withdrawal of 30mg ETP	6.09^{a}±0.31	4.94^{a}±0.17	6.89^{a}±0.13	5.54^{a}±0.53	11.78^{a}±0.27	41.63^{a}

[Values having different superscripts (a, b, c) within column differ significantly ($P<0.05$)]

Cell type morphology of estrous cycle

Morphological changes in different cell types of estrous phases were observed in chemical treated groups throughout the experimental period as compared with control group (Fig. 1). Enlargement in sizes of cornified cells along with the reduction in their number were noted in the estrous phase in earlier days of treatment. The reduction pace in the number of cornified cells remain continued till the end of experiment, whereas their cell sizes were reduced in association with nuclear and cytoplasmic degeneration mainly in high dosed group that was prominent in the later part of exposure paradigm (i.e., 90 days). On the other hand, the epithelial cells showed elevation in their number with attainment of enlarged sizes. Bulging of epithelial cells with hyperchromic nucleus were noted extensively in all the treated groups in the later days. The leucocytes remain unchanged in their morphology, although their number increased throughout the period of exposure paradigm. In BS treated groups, the effect of ETP treatment was shown to be reduced as the morphological alterations in cells of different estrous phases were reduced and close to control group. The groups IV and V showed a lower number of abnormal cells, and the percentage of abnormal cells was closer to that of the control group. Details of numerical changes in morphological alterations of cell types of estrous phases in treated and control groups are shown in Table 2.

Weight of male reproductive organs

Analysis of weight of male reproductive organs *viz*. testicular and accessory sex organs and their relative individual weight along with sperm dynamics have been used as important marker for manifestation of male reproductive toxicity caused by chemicals. A gradual decrease in weight of testis and accessory sex organs such as epididymes and vasa-deferentia and their relative weights were observed in both treated groups, which were highly significant in higher dosed group i.e. 30mg/kg body wt when compared with low dose and control. However, 15mg group also showed reduction in organ weight and their relative weight was also significant (P<0.05) as compared to control group. In BS treated groups, the relative organ weight of the testicular tissues increased than those of the ETP treated groups and showed no significant (P<0.05) difference from the control group as shown in Table 3.

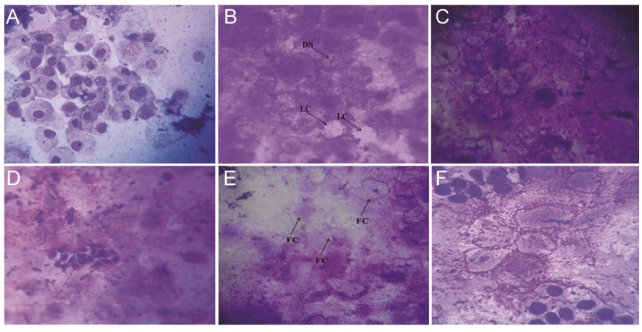

Fig. 1 — (A) Normal cellular structure of estrous cell types in control rats, (B) Cells with degenerated nucleus (DN) and Loss of cytoplasm (LS) in ETP treated rats of higher dosed group (30 mg/kg bw), (C) Cells with hyperchromic (HN) and degenerated nucleus (DN) in estrous cycle of treated rats of lower dosed group (15 mg/kg bw), (D) Accumulation of lymphocytes during estrous phases in ETP treated rats of 30 mg/kg bw group, (E) Cornified cells with foamy cytoplasm in ETP treated rats of 15 mg group, (F) Cells with protective effect of BS on degenerating membrane (DM) and hyperchromic nucleus (HN) in cell types of estrous phases of ETP treated rats, [×40].

Effect of ETP on semen parameters

Sperm motility, viability and sperm count

Chronic administration of ETP at a dose of 15mg/kg body wt and 30mg/kg body wt showed a significant (P<0.05) progressive decrease in the sperm motility compared to the control group. Although, the trend of decreased level occurred in a dose dependent and time dependent manner as shown in Table 4.

A significant reduction in the percentage viability (ratio of live and dead sperms) was recorded in both the ETP treated groups compared to the control group at 30 days of exposure. On the other hand, a significant reduction (P<0.05) in the percentage of sperm viability in both treated groups was noted in later days of exposure i.e. from 60 days onwards in comparison to control group (Table 4).

An insufficient number of sperm count was observed in the sperm collected from cauda epididymal region of all the rats exposed to different doses of ETP throughout the experimental period when compared with control rats. The mean sperm count per epididymal volume of rats administered with 15mg/kg and 30mg/kg body weight of ETP was found to be progressively reduced in number and was significant (P<0.05) as compared to control group. However, a highly significant decrease in sperm density was noted in the later days of exposure paradigm in all the treated groups for both the doses. Although sperm density did not show much variation in both the treated groups in earlier days of exposure but much variation in sperm count was recorded in both low (15mg/kg body wt) and high (30mg/kg body wt) dose exposed rats at the later part of 60 and 90

Table 2 — Impact of BS in ETP induced effects on morphological alterations of different estrous cell types and their numerical changes

Estrous phases	Groups	Duration of treatment																							
		30 days								60 days								90 days							
		TC	NC	AC	MD	CD	ND	AS	AC%	TC	NC	AC	MD	CD	ND	AS	AC%	TC	NC	AC	MD	CD	ND	AS	AC%
Proestrus (mostly epithelial, few corniffied cells)	Group I (control)	50	46	4	-	1	-	3	8	50	45	5	1	2	1	1	10	50	46	4	1	-	1	2	8
	Group II (15mg)	50	37	13	3	1	-	9	26	50	31	19	8	1	4	6	38	50	27	23	6	2	4	11	46
	Group III (30mg)	50	32	18	5	3	-	10	36	50	27	23	7	-	4	12	46	50	21	29	7	2	7	13	58
	Group IV (100mg) BS extract after withdawal of 15mg ETP	50	44	6	1	1	-	4	12	50	43	7	3	2	1	2	14	50	45	5	-	1	2	3	10
	Group V (100mg) BS extract after withdrwal of 30mg ETP	50	46	4	2	-	-	2	8	50	47	3	2	1	-	1	6	50	48	2	1	-	1	2	4
Estrus (mostly cornified cells)	Group I (control)	50	47	3	1	2	-	-	6	50	46	6	1	3	-	2	12	50	46	4	-	2	1	1	8
	Group II (15mg)	50	39	11	6	1	-	4	22	50	29	21	7	3	-	11	42	50	26	24	11	8	1	4	48
	Group III (30mg)	50	33	17	5	2	1	9	34	50	24	26	9	4	3	10	52	50	19	31	11	7	1	12	62
	Group IV	50	48	2	-	1	-	1	4	50	45	5	1	3	-	2	10	50	46	4	3	1	-	1	8
	Group V	50	49	1	-	-	-	1	2	50	47	3	1	1	-	1	6	50	44	6	3	1	2	1	12
Metestrus (mostly leucocytes and few cornified cells	Group I (control)	50	46	4	-	2	2	-	8	50	48	2	-	-	1	1	4	50	43	7	2	1	2	2	14
	Group II (15mg)	50	40	10	3	-	6	1	20	50	39	11	1	1	5	4	22	50	35	15	2	2	5	6	30
	Group III (30mg)	50	41	9	1	2	4	2	18	50	40	10	1	-	5	4	20	50	36	14	3	-	7	4	28
	Group IV	50	47	3	-	2	-	2	6	50	45	5	2	1	-	3	10	50	44	6	2	2	1	3	12
	Group V	50	49	1	-	1	-	1	2	50	47	3	1	-	-	3	6	50	46	4	1	-	1	2	8
Diestrus (mostly leucocytes and a few epithelial cells)	Group I (control)	50	49	1	-	-	1	-	2	50	45	5	1	1	2	1	10	50	49	1	1	-	-	-	5
	Group II (15mg)	50	42	8	1	-	5	2	16	50	38	12	2	-	7	3	24	50	37	13	1	1	5	6	26
	Group III (30mg)	50	39	11	2	1	6	2	22	50	34	16	2	1	8	5	32	50	31	19	2	2	8	7	38
	Group IV	50	49	1	1	-	-	-	2	50	45	5	1	-	1	2	10	50	48	2	-	-	2	2	4
	Group V	50	47	3	1	-	1	3	6	50	47	2	1	2	1	2	6	50	49	1	-	1	-	1	2

[TC = Total cells counted, NC = Normal cells, AC = Abnormal cells, MD = Membrane deformities, CD = Cytoplasmic disintegration, ND = Nuclear deformities, AS = Alteration of size, AC% = Abnormal cell percentage]

days of experiment. However, the decreased response in sperm density was dose and time dependent in all the cases.

In case of BS treated groups, the decrease in the sperm motility caused by administration of ETP was reduced and the results showed no significant difference from that of the control group (P<0.05). The percentage viability of the sperm cells were also found to increase in the BS treated groups. The sperm count showed increase in sperm density in the BS treated groups than those of the ETP treated groups and the results were similar to the control group with no significant difference (P<0.05) (Table 4).

Sperm cell morphology

Both the ETP treated groups of rats showed some gross morphological deformities that vary from head piece region to tail region compared to normal sperm of the control groups (Fig. 2). The most common sperm deformities noted were head deformities such

Table 3 — Body weight, organ weights (testis and accessory sex organs) and their relative weights in control, ETP treated groups and BS treated groups after withdrawal of chemical treatment (values are mean ± SE of 5 animals)

Days of treatment	Groups	Body weight (gm)	Organ weight					
			Cauda epididymes (mg)	Relative weight (%)	Vas deferens (mg)	Relative weight (%)	Testis (mg)	Relative weight
Control	Group I	134.87^{a}±11.19	709.83^{a}±12.31	0.53^{a}	167.41^{a}±6.87	0.12^{a}	1891.12^{a}±21.01	1.40^{a}
30 days	Group II	140.42^{a}±21.11	683.23^{a}±15.09	0.49^{a}	160.13^{a}±7.23	0.11^{a}	1847.43^{a}±23.04	1.32^{a}
	Group III	141.12^{a}±21.11	642.23^{a}±15.09	0.46^{a}	155.13^{b}±7.23	0.11^{a}	1793.43^{b}±23.04	1.27^{b}
60 days	Group II	137.92^{a}±15.12	598.32^{b}±14.63	0.43^{b}	146.56^{b}±5.18	0.11^{a}	1735.19^{b}±17.50	1.26^{b}
	Group III	135.86^{a}±23.17	542.67^{c}±9.87	0.40^{c}	140.09^{b}±4.36	0.10^{b}	1645.38^{c}±25.37	1.21^{c}
90 days	Group II	134.92^{a}±20.05	529.76^{c}±10.61	0.39^{c}	129.67^{c}±5.28	0.10^{b}	1591.23^{c}±19.17	1.18^{c}
	Group III	136.75^{b}±11.57	468.41^{c}±13.67	0.34^{c}	121.78^{c}±7.21	0.09^{c}	1483.76^{c}±30.68	1.09^{c}
90 days	Group IV	132.68^{a}±9.31	693.20^{a}±13.09	0.52^{a}	161.17^{a}±5.86	0.11^{a}	1852.18^{a}±19.03	1.37^{a}
	Group V	134.30^{a}±12.5	655.70^{a}±11.33	0.41^{a}	159.27^{b}±6.56	0.09^{a}	1789.49^{a}±20.18	1.30^{a}

[Values having different superscripts (a,b,c) within column differ significantly (P<0.05)]

Table 4 — Ameliorative activity of BS in the ETP induced stress on semen parameters of treated and control group rats (values are mean ± SE of 5 animals)

Days of treatment	Groups	Sperm motility (%)	Live/ Dead Ratio (%)	Sperm Count (x10^{6}/ml)
Control	Group I	80.3±0.72^{a}	91.78±1.63^{a}	88.02±1.01^{a}
30 days	Group II	63.23±2.38^{b}	89.82±2.58^{a}	69.01±2.38^{b}
	Group III	59.67±1.11^{b}	88.23±1.78^{a}	61.50±3.19^{b}
60 days	Group II	49.51±0.89^{b}	82.73±2.08^{b}	56.50±0.95^{c}
	Group III	39.27±1.19^{c}	79.28±3.37^{b}	43.14±1.53^{c}
90 days	Group II	37.12±2.10^{c}	79.13±2.54^{b}	43.83±1.19^{c}
	Group III	28.30±3.01^{c}	70.53±3.32^{b}	29.95±2.01^{c}
90 days	Group IV	75.31±0.81^{a}	86.20±2.11^{a}	85.57±1.73^{a}
	Group V	78.5±0.69^{a}	88.70±1.08^{a}	90.05±2.01^{a}

[Values having different superscripts (a, b, c) within column differ significantly (P<0.05)]

as damaged head, head without acrosomal hook, duplication of head, headless sperm and tailless sperm (Fig. 2). Various tail defects like breakage and curved tail, rudimentary and coiled tail were noted in chemical treated group (Fig. 2). Whereas mid piece showed banding form. In some cases, an unusual (1-3 in numbers) cytoplasmic droplets were marked feature that were found to be associated with outer membrane sheath of different parts of sperm as a residual body. In the BS treated groups, the various morphological defects shown by the sperm head and tail regions were lesser in number than that of the chemically treated groups. The number of abnormal sperm cells were also reduced and similar to the control groups, in case of group IV and V. The details of effect of BS on different types of sperm morphological deformities are shown in Table 5.

Effect of ETP on male fertility

The effect of ETP on male fertility aspects such as litter weight, number of litter delivered and percentage of fertility are shown in details in Table 6.

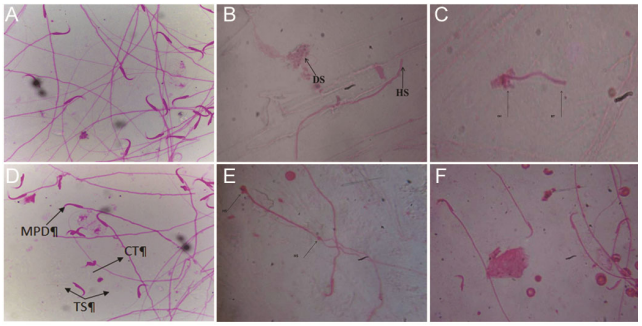

Fig. 2 — (A) Normal morphology of the sperm from the control group, (B) Headless sperm (HS) and a degenerating sperm (DS) from ETP treated groups exposed to high dose, (C) Sperm with damaged head (DH) and breakage tail (BT) from ETP treated groups exposed to high dose, (D) Mid piece defect (MPD), tailless sperm (TS) and curved tail (CT) from ETP treated groups exposed to high dose (90 days), (E) Impact of BS on the headless sperm (HS) from ETP treated groups (30 mg/kg bw), (F) BS activity showing normal morphology of the sperm after withdrawal of ETP (30 mg/kg bw), [100×].

The control female rats used for mating with the control male produced litters having an average weight 7.38±0.21 gm whereas an average weight of 5.04±0.19 gm and 4.93±0.05 gm of litters were produced by female rats which mated with ETP treated male group of 15mg/kg body weight and 30mg/kg body weight, respectively. The litter weights of ETP treated groups were decreased which is statistically significant (P<0.05) as compared to control ones.

Similarly, female rats used for mating with control male group produced an average number of 9.02±0.50 litters. An average litter number was found to be decreased in all the female rats used for mating with ETP treated male rats (15mg/kg body weight and 30mg/kg body weight) and were significant (P<0.05) when compared with control ones.

Further, the fertility percentage of normal female

Table 5 — Impact of BS in ETP induced morphological abnormities of sperm in the treated groups compared to control groups

Duration of exposure	Groups	Total no of cells counted	Normal cells	Abnormal cells	Tail deformities				Head deformities					Mid piece deformities	Cytoplasmic droplet as residual body	Abnormal cell (%)
					CT	BT	RT	CoT	DH	HAH	DuH	HS	TS			
Control	Group I	200	183	17	3	2	2	1	3	1	-	2	1	1	-	8.5%
30 days	Group II	200	171	29	7	1	2	3	4	2	1	4	3	1	1	14.5%
	Group III	200	162	38	6	4	1	2	8	2	-	5	5	3	2	19%
60 days	Group II	200	148	52	9	4	7	4	10	5	3	4	3	1	2	26%
	Group III	200	129	71	18	8	7	3	15	4	2	7	5	1	1	35.5%
90 days	Group II	200	114	86	17	7	5	3	21	4	2	11	9	3	4	43%
	Group III	200	89	111	23	14	2	2	27	3	1	19	11	6	3	55.5%
90 days	Group IV	200	189	11	2	-	1	2	2	-	1	1	1	-	1	5.5%
	Group V	200	182	18	4	2	3	-	4	2	1	-	-	-	2	9.0%

[CT- Curved Tail, BT-Breakage Tail, RT- Rudimentary Tail, CoT- Coiled Tail, DH- Damaged Head, HAH- Head without Acrosomal Hook, DuH- Duplication of head, HS- Headless Sperm, TS- Tailless Sperm]

Table 6 — Fertile capability in control, ETP treated and BS treated male rats (values mean ±SE of 9 animals)

Groups	Litter weight (gm)	Number of litter delivered	Percentage of fertility (%)
Group I	7.38±0.21[a]	9.09±0.50[a]	100[a]
Group II	5.04±0.19[b]	4.85±0.35[b]	56[b]
Group III	4.93±0.05[b]	2.35±0.01[b]	22[c]
Group IV	6.8±0.17[a]	9.20±0.35[a]	99[a]
Group V	6.97±0.30[a]	8.92±0.43[a]	98[a]

[Values having different superscripts (a, b, c) within column differ significantly (P <0.05)]

rats used for mating with control male group showed 100% fertility rate. While both ETP treated male (15mg/kg body weight and 30mg/kg body weight) used for mating with normal female rats showed 56% (Group III) and 22% (Group IV) fertility rate. The fertility rate was found to be reduced in both the chemical treated groups and differed significantly (P<0.05) from control group.

For groups post-treated with BS extract, the litter weight was found to increase more than that of those treated with ethephon alone. The number of litter delivered also increased and was almost equal to the control group. Furthermore, the fertility percentage in BS treated extract was 99% in group IV and 98% in group V, which were much higher than in groups II and III. The effect on fertility percentage caused by ethephon exposure was found to be reverted by use of BS extract. The litter weight, number of litter delivered and percentage of fertility showed no significant difference from the control group (P<0.05).

Histological studies

The effect of ETP exposure in the reproductive organs of both genders were clearly visible after performing histological analysis of testis and ovary of the groups II and III. The testis of group II showed loss and partial degeneration of spermatogonial cells with vacuolation, absence of primary and secondary spermatocytes, lysis and necrosis of spermatogonial cells. The testis of group III showed reduction in testicular tubules, profused vacuolation and total loss of intertubular connective tissue associated with necrosis and degeneration of Leydig cells showing inhibitory effect on testicular tissue (Fig. 3). Treatment with BS extract after withdrawal of ETP treatment showed a reduction in the effect of ETP in the testicular and ovarian tissues of group V. The testis of group V showed a normal histo-architecture just like the control group. It also showed actively dividing germinal epithelium with secondary spermatocytes. In ovary sections of Group I normal structures such as oocyte, zona pellucida layer, stroma *etc.* were seen (Fig. 3). The effect of ETP treatment is well observed in Group II and III where, degenerated oocyte is seen as well as no distinct germ layer is observed (Fig. 4). Administration of BS extract helped recovering some of the damaged caused by exposure to ETP, as seen in the ovarian tissue of Group V (Fig. 4).

Study of antioxidant enzyme activities

After 90 days treatment with ETP, the results of antioxidant enzyme activity assay showed a significant increase in the level of MDA and other antioxidant enzymes in the testicular tissue in both the chemically treated groups. Increase in MDA level and SOD, GPx and CAT activity in group II were significantly more than that of the control group (P<0.05). In group III, increase in MDA level was significant at P<0.05 and increase in level of SOD, GPx and CAT activities was significant at P<0.01. In the BS treated groups, the activities of antioxidant enzymes CAT and SOD, were slightly increased but

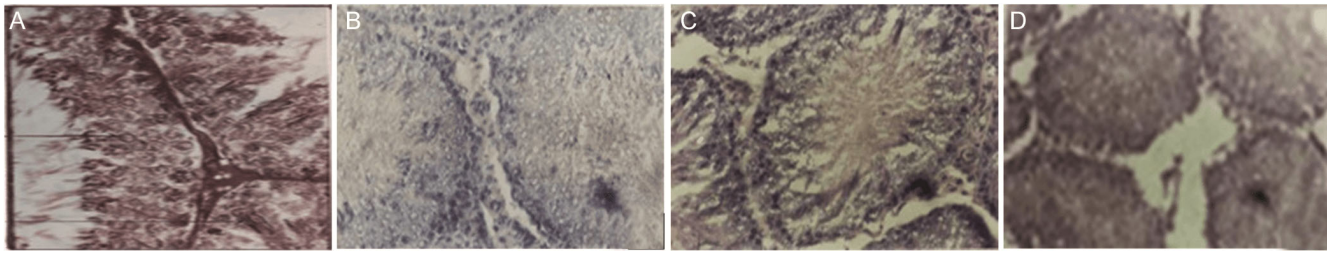

Fig. 3 — (A) T.S. of control, (B) BS treated testis showing actively dividing germinal epithelium with secodary spermatocytes and sperm in abundance, (C) T.S. of testis exposed to 15 mg/kg bw of ethephon for 90 days showing loss and partial degeneration of spermatogonial cells with vacuolation, absence of primary and secondary spermatocytes, lysis and necrosis of spermatogonial cells, (D) T.S. of testis exposed to 30 mg/kg bw of ethephon for 90 days showing reduction in testicular tubules, profused vacuolation and total loss of intertubular connective tissue associated with necrosis and degeneration of Leydig cells showing inhibitory effect on testicular tissue, [40×].

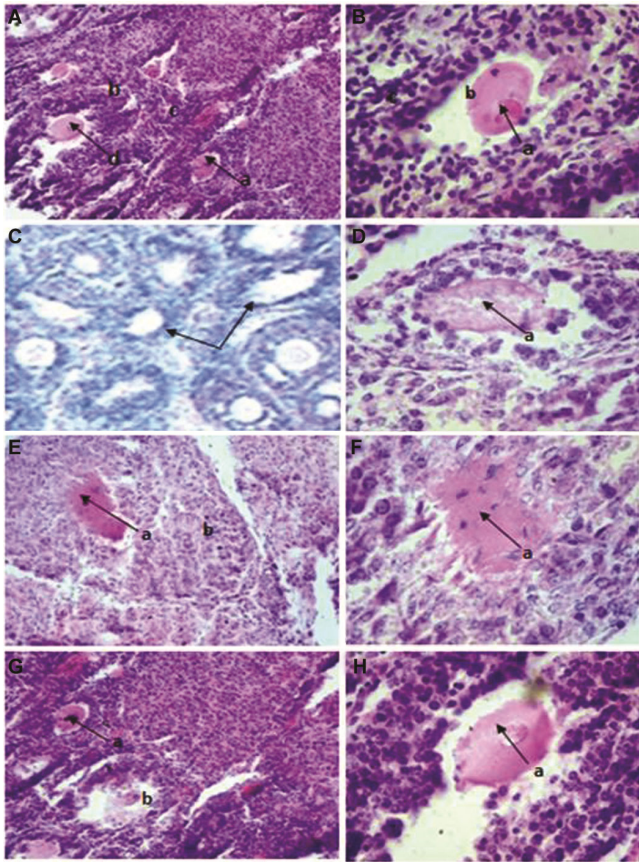

Fig. 4 — Normal histo-architecture of ovary of control group, (A) Normal oocyte [a & d], Graafian follicle [b] and stroma [c]; (B) Normal oocyte [a], zona pellucida [b] and granulosa cells [c] are seen; Toxic effect of ETP exposure in ovary of treated group (C) 15 mg/kg bw ETP treated ovary showing many Atretic follicles; (D) 15 mg/kg bw ETP treated ovary showing oocyte degeneration [a]characterized by the disintegrated zona pellucid and germinal layer of the follicle; (E) 30 mg/kg bw ETP treated showing degenerated oocyte [a] and disintegrated germinal epithelium [b]; (F) 30 mg/kg bw ETP treated ovary degenerated oocyte [a] characterized by indistinct germinal epithelium; Protective effect of B.S treatment after withdrawal of ETP, (G) BS treated ovary showing a normal oocyte [a], stroma [c] and little damaged Graffian follicle [b]; (H) B.S. treated ovary showing normal oocyte [a] and reduction in changes occurred due to ETP treatement.

the levels were not significantly different from the control group. In group IV, the MDA level was slightly reduced than the control group but the results were not statistically significant. In both the BS treated groups, GPx activity were slightly reduced but the results were not significantly different from that of the control group (Table 7).

Discussion

ETP, an organophosphorous pesticide, is widely used as a plant growth regulator, and it contains chloride and phosphate in its chemical structure. In the tissue of plant and animal, ETP can be converted into ethylene gas and chloride[19]. Further, ethylene can be converted to ethylene oxide, then to ethanediol and hydroxyethyl- glutathione and marcaturic acid. Ethephon has been reported to inhibit cholinesterase enzyme activity. Because of these changes different disorders like reproductive system impairment can occur[20,21].

The result of the present study revealed deleterious responses of ETP on the reproductive functions of albino rats of both sexes. The effect was found to be varied with regard to different doses and duration of exposure. The histoarchitecture of both testis and ovary were found to be affected after the exposure to ETP. Results were similar with previous studies. In one of the studies, it was seen that ETP exposure led to reduction in germinal epithelium height, seminiferous tubules in the testis of ethephon treated male mice[22]. Another study done on mice model showed that ethephon intoxication causes decrease in serum levels of female hormones and has a negative role in female reproductive parts[23]. Similar studies done with another organophosphate insecticide diazinon in rat model resulted in reduction in the mean number of corpus luteum of the ovary of treated groups in the female rats[24]. Present investigation, the

Table 7 — Efficacy of BS in ETP induced effect on the MDA level and antioxidant enzyme activities in the testes of control and treated groups.

Biochemical parameters	Group I	Group II	Group III	Group IV	Group V
MDA level (units/n-mole/mg of protein)	1.3±0.56	2.9±0.92*	4.2±0.63*	1.2±0.46	1.5±0.26
SOD (units/mg of protein)	13.33±2.08	21.95±1.35*	27.96±2.12**	14.15±1.30	15.01±2.60
GPx (units/mg of protein)	0.59±0.06	1.10±0.09*	1.97±0.04**	0.48±0.05	0.52±0.03
CAT (units IU/mg of protein)	23.29±2.35	26.2±1.6*	38.47±2.88**	23.3±1.5	25.1±1.9

[Compared to control groups (*P<0.05, **P <0.01)]

impact of ETP manifested functional disturbances in certain reproductive aspects of albino rats. Responses to ETP exposure in different groups of rats were found to be non-uniform. Reduction in body weight was observed in both sexes treated with 15mg and 30mg/kg body weight but reductional pace in the body weight was more prominent in the male groups exposed to high dose (30mg/kg body weight) as compared to female rats. This may be occurred on account of ETP induced toxic in a graded manner. Similarly, food consumption rate was reduced in ETP exposed groups that indicated disturbances in metabolic processes and in turn led to impairment in normal pace of body weight gain. This is in close consortium with earlier reports of a study conducted by Miller and Troup[25].

Present study showed that exposure to ETP causes gradual decrease in weight of testes and accessory reproductive organs such as epididymis and vas deference and their relative body weights in both treated groups. This result is similar to the early findings of Joshi *et al*., where male rats were exposed to an organo-phosphorous pesticide, methyl parathion at a dose of 30 mg/kg body weight for 30 days which manifested and a significant decrease in the weight of the testis, seminal vesicle, epididymis and ventral prostate was observed[26]. Similar results with a significant reduction in the weight of testes and accessory sex glands on male rats exposed to an another organophosphorous insecticide, quinalphos also supported the present findings[27]. The present study suggested that chronic administration of ETP decreased sperm count, sperm motility and caused various morphological sperm abnormalities. Eldaim *et al*., reported ethephon intoxication reduced motility percentage, sperm count and sperm vitality which is also in agreement with present study[28].

In the present investigation, the fertility percentage was decreased in both ETP treated groups in a dose dependent and time dependent manner (15 mg/kg body weight and 30 mg/kg body weight) as compared to the control group. This is because ethephon can cause damage to sperm DNA and gradually decrease the sperm quality and fertilization potential[29]. Biswas *et al.*, have observed same effect of ETP on male reproductive system[30]. Damaged testicular cell′s DNA and Chromatin suppress the expression of PCNA (Proliferating cell nuclear antigen) and induce the overexpression of p53 proteins in testicular tissue. It resulted suppression of spermatogonia proliferation and increase the percentage of abnormal sperm. Balkanloo *et al*., 2020 have found similar effect of ethephon on female reproductive system of mice[31]. It was concluded by them that ethephon induces oxidative stress which can cause detrimental effect on reproductive system and embryonic development in mice.

Due to exposure to ETP at the dose of 15mg/kg bw and 30mg/kg bw a significant change in estrous cycle with decreases in the phases of estrous cycle especially proestrus, estrous and metestrous and a concomitant increase in duration of diestrus phase was observed. This revealed that ETP induced stress caused impairment of reproductive potential with subsequent increase in the risk of infertility in female. The finding is in agreement with report of Hamdani *et al*., where there was a reduction in estrous periodicity of progeny of Cypermethrin (2.76 mg/kg bw and 5.52 mg/kg bw) treated mice[32]. A similar work reported in mice treated with carbofuran, an insecticide, causes a significant decrease in the duration of proestrus, estrous and metestrous with a significant increase in diestrus phase also supported the present study[33]. The changes in estrous cycle as observed in the present findings may be due to alteration in serum levels of sex hormones caused by ETP induced toxic stress. It was showed that chronic exposure to ethephon may cause negative effects on the reproductive system of female by gradual reduction in the serum progesterone and estradiol along with elevated levels of FSH and LH[23].

ETP at dose 15mg/kg bw and 30 mg/kg bw showed a decrease in the number of cornified cells along with their enlargement in sizes at the early days of the

experiment. Whereas size of the cornified cells were found to be reduced in high dosed group in the later part (i.e., 90 days) of experiment paradigm. There was an increase in the number of epithelial cells with enlarged sizes and the leucocytes remain unchanged in their morphology, but their number increased throughout the experiment. A wide number of evidences suggested that vaginal smear from rats treated with imidacloprid, an insecticide, showed increased number of nucleated epithelial cell during proestrus, a large number of cornified epithelial cells during estrous. In contrary, a small number of cornified epithelial cells and a small number of nucleated epithelial cells during metestrus and diestrus phases, respectively[34].

The reports of earlier investigations revealed that various herbal medicines can be used to mitigate tissue damages caused by chemical agents induced toxic stress. Bamboo shoot has been used as traditional medicine since 2005 especially in China. In North-East India bamboo shoot is eaten medicinally by different tribes such as Tiwa, Naga, Mizo *etc*.[35] It has also been a part of regular cuisine of South Asian countries, including India in several food preparations due to its good taste and rich nutrient content[36]. Bamboo shoot is rich in protein, dietary fiber, vitamins, phenols, and phytosterols. Dietary fibre possesses several health benefits and it controls blood pressure, obesity and also protects our body from cardiovascular diseases and potential carcinogens. It also has antioxidant, anti-inflammatory, antimicrobial, antihelminthic, antidiabetic and anti-ulcer activities. Some of the species of bamboo shoots interfere in male and female reproductive functions whereas some reports showed a positive impact in this regard.[37,38,39,40] Present study demonstrated that oral administration of shoot extract of *Bambusa balcooa* after withdrawal of ethephon ameliorated the toxic stress caused by ethephon such as oxidative stress and gonadal function in albino rat.

SOD and CAT play important role in maintaining cell homeostasis by ROS scavenging. SOD catalyzes dismutation of superoxide radical into hydrogen peroxide (H_2O_2) and molecular oxygen where as CAT catalyzes the decomposition of H_2O_2 to water and oxygen. Glutathione related enzymes like glutathione peroxidase (GPx) shows antioxidant activity directly or indirectly. Malondialdehyde (MDA) is the final product of polyunsaturated fatty acids peroxidation in the cells and increased MDA content is a sign of lipid peroxidation.[41,42,43] In the present study, there was a significant increase in the levels of SOD, CAT, GPx and MDA in testicular tissue, reflects disturbances in normal oxidative mechanism during ETP toxicity. However, oral administration of *Bambusa balcooa* shoot extract to ETP treated mice showed a partial recovery in the above mentioned parameters in a dose-dependent manner. In accordance with our findings, Goyal *et al*., 2017 stated that treatment with *Bambusa balcooa* leaf extract help in reducing oxidative stress by removing free radicals in alloxan-induced diabetic rats[44].

From the present study, it was found that shoot extract of *Bambusa balcooa* enhances sperm quality, sperm motility and sperm count in male rat by counteracting the toxic effect of ETP. After administration of shoot extract of *Bambusa balcooa*, body weight, and other organ weight *viz*. epididymis and vasa deferentia and their relative weights became almost normalized to their control counter parts. This may be due to subsided toxic effect of ETP by antioxidant and anti-inflammatory activities of BS extract. The similar result was found from the work done by Sumedha *et al*.[45] It was further reported by him bamboo (*Dendrocalamus strictus*) seed extract of other species can remove toxic effect of endosulfan, a pesticide and induce fertility in male rat. The mechanism behind the effects of bamboo shoot extract on the reproductive system may be correlated with the development of hormonal balance in LH, FSH and testosterone profiles along with its antioxidant activity. Further reports showed that bamboo seed extract increased the secretion of testosterone that increased fertility of the sperm[46]. There were some contradictory results that reported the presence of antifertility potential in different parts of bamboo plants[47,48].

Shoot extract of *Bambusa balcooa* also reduced the toxic effect of ethephon on female reproductive system. Rats treated with ethephon showed irregularity in estrous cycle and after exposure of bamboo shoot extract the irregularity in estrous cycle changed to normal sequence. This is because shoot extract of *Bambusa balcooa* may have gonadotropic function which can activate hormonal profiles and stimulate regeneration of ovarian follicles and increased the production of estrogen which will result in regularity of estrous cycle. Similar work with similar result by Soujanya *et at.*, (2022) with BS extract by treatment in rats has also been reported[34]. A

work conducted by Soumya *et al*., 2016 manifested the role of bamboo seed oil (*Bambusa bambos* Druce) in restoring of estrous cycle in letrazole induced polycystic ovarian disease in female rats[49]. These results are different from the findings reported by Sarkar *et al*., 2017 where consumption of bamboo shoots decreased ovarian as well as uterine weight, decreased estradiol, estriol and progesterone levels of female rats[50]. This may be due to cyanogenic constituents of bamboo shoot that could impact fertility[51].

Conclusion

From the present investigation, it can be concluded that the shoot extract of *Bambusa balcooa* has the potential to mitigate gonadal disorders caused by oxidative stress after ethephon exposure in the bodies of male and female Wistar rats. Extract of *Bambusa balcooa* shoot reduced the detrimental effect of ethephon on the reproductive organs as well as different parameters indicating reproductive health such as enhanced sperm motility, viability in males as well as regulated estrous cycle and improved litter production in females. This may be attributed to the presence of less cyanogenic activity which may have been caused by cleansing of bamboo shoot which is done before performing the experiment or high antioxidant activity of *Bambusa balcooa* shoot extract. The results of this study prove the gonadotropic function of *B. balcooa* which may lead to fertility enhancing effects in mammals suffering from gonadal disorders.

Ethical statement

The experiment was carried out according to the guidelines of CPCSEA and after approval of Institutional Animal Ethical Committee vide certificate of approval 8/IAEC/CU/05/01/2021.

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Conflict of interest

There was no conflict of interest declared by the authors.

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