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Ameliorative Potential of Wheatgrass against Histological and Biochemical Alterations caused due to Chlorantraniliprole and Arsenic in Female Albino Rats

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***Abstract*:** The ameliorative potential of wheatgrass juice was evaluated against the reproductive toxicity caused by combination of pesticide, chlorantraniliprole and heavy metal, arsenic in the female albino rats to address the growing concerns about simultaneous exposure to agrochemicals and heavy metals in the environment and assess their impact on female reproductive function. The rats were administered with chlorantraniliprole (1/2, 1/4 and 1/6 LD_{50}), arsenic(0.50 mg/L of water) and fresh wheatgrass juice (10 ml/kg b.w.) orally. The significant alterations such as increase in duration of estrous cycle, reduced number of follicles, increased atresia (%) in the ovaries, decreased diameter of uterine epithelium, myometrium and endometrium and scattering of lumen in the uterus; reduction in the protein and lipid content were observed in chlorantraniliprole (1/2, 1/4 LD_{50}) + arsenic treated rats as compared to control rats. However, less significant alterations were observed in the reproductive parameters of the rats treated with groups exposed to chlorantraniliprole (1/6 LD_{50})+ arsenic and only arsenic when compared to the control group. These changes were reduced with administration of fresh wheatgrass juice thus suggesting that wheatgrass acts as an ameliorative agent against chlorantraniliprole and arsenic induced female reproductive toxicity rats.

Keywords: Arsenic, Antioxidants, Biomarker analysis, Chlorantraniliprole, Wheatgrass.

1. INTRODUCTION

Wheat (*Triticum aestivum L.*) is one of the most commonly grown crops in India. The germination of wheat produces wheatgrass after 6-10 days which is rich in vitamins (A, B, C, E), chlorophyll, minerals, flavonoids, phenolics and enzymes like SOD, cytochrome oxidase that contribute to the therapeutic potential of wheatgrass (Tamraz et al., 2024). Wheatgrass is widely recognized for its anticancer potential, owing to its high chlorophyll content along with vitamin E, B and other essential minerals, that add to its medicinal value (Mishra et al., 2025). Since the atomic structures of chlorophyll in wheatgrass and haemoglobin in blood are nearly identical, except that magnesium is the central element in chlorophyll, while iron occupies this position in haemoglobin, this similarity allows wheatgrass to restore the body's alkaline balance, reduce acidity and promote the formation of healthy cells (Sharma et al., 2025). Wheatgrass also contributes to improved fertility and chances of pregnancy in women (Kaur et al., 2023). To satisfy the demands of the expanding population, agricultural production and use of pesticides and insecticides for reducing pest populations has been expanded in recent years which severely impacted the agricultural system (Bitschinski et al., 2024).

A new class of insecticides known as the Diamide Group-28 insecticides, was recognised in March 2010 under the Insecticide Resistance Management Global Guidelines of the Insecticide Resistance Action Committee (Du and Hu, 2023). These insecticides would target particular ryanodine receptors in insects, which by interacting with the calcium channels of the insect's cardiac and skeletal muscles impede the transmission of nerve impulses and offer protection (Ponepal et al., 2023). Chlorantraniliprole is applied on large number of crops such as brinjal, cotton etc. against pest species attacking these crops such as Lepidoptera. Chlorantraniliprole has a short half-life, its LD_{50} of 5000 mg/kg of body weight as per the USEPA exerts toxic effects on mammalian health (Wang et al., 2025). However, the environmental residues of chlorantraniliprole have been reported in crops such as rice, tomato, sugarcane and cotton (Mahato et al., 2023). Chlorantraniliprole accumulates in the muscle tissue of fishes and degenerates the detoxification pathways (Yin et al., 2023). It leads to an imbalance between pro-oxidants

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and antioxidants leading to severe reproductive diseases such as endometeriosis, polycystic ovarian syndrome etc. (Omar et al., 2022). Its exposure leads to severe damage to various body organs like kidney, liver, lungs etc. and causes neurological health effects including memory or learning disability, vision, impairment, signalling disability etc. along with causing immunological disorders such as hypersensitivity, asthma, and allergic reaction (Kimura et al., 2023). Its intrauterine exposure during pregnancy exerts its teratogenic effect on foetuses along with retarded morphological and mental growth (Kaabeche et al., 2024).

Heavy metals are metallic chemical elements having density higher than the density of water. Industrialization and urbanization have also led to an increased anthropogenic contribution of heavy metals within the environment (Kapoor et al., 2024). Arsenic (As) is one such heavy metal that is prevalent in water at concentrations higher than the permissible limit i.e 10 µg/L as recommended by WHO in most parts of the World with East Asian Countries being the most affected with over 230 million people at risk of drinking water exceeding the World Health Organization's (WHO) guideline of 10 µg/L (0.01 mg/L) (Shaji et al., 2021). Around 220 million individuals worldwide, particularly in Asia, may consume water with Arsenic above permissible limits (Jha et al., 2023). It has been identified as a carcinogen by the International Agency for Research on Cancer (IARC) for human skin, bladder and lungs. Arsenic exposure increases the evidence of stillbirth and even infertility in females due to the generation of free radicals. Arsenic owing to its lipophilic property gets bioaccumulated in organisms and is responsible for disrupting the biochemical and physiological functions in mammals (Mouttoucomarassamy et al., 2024; Ramesh, 2024).

The studies have been already conducted on the toxic effects on various systems being caused by chlorantraniliprole and arsenic individually as well as the protection provided by the wheatgrass in the various therapeutic studies in rats as a mammalian model (Mahboob et al., 2025; Mahmoud et al., 2025 and Raslan et al., 2025). However, no study has reported the combined toxic effect of chlorantraniliprole and arsenic on mammalian reproductive health and its possible amelioration with a natural herb such as wheatgrass. The current study therefore evaluates the ameliorative potential of wheatgrass against combined reproductive toxicity caused by the interaction of variable doses of chlorantraniliprole along with a fixed dose of arsenic by determining the alterations in the various parameters of female albino rats.

2. MATERIALS AND METHODS

2.1. Procurement of Animals

The sexually mature female albino rats were procured from the Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar after clearing the proposal through Institutional Animal Ethics Committee (IAEC), GADVASU. The rats were acclimatized for 10 days before using them for experimentation. The rats were maintained under controlled conditions of temperature (22±2°C) and provided with standard diet containing pelleted food and water. The experimental protocol met the National guidelines on the proper care and use of animals in the laboratory research. The Institutional Animal Ethics Committee (IAEC) vide letter no GADVASU/2022/IAEC/63/11 dated 13/01/2022 approved this experimental protocol.

2.2. Treatment of Animals

Wheatgrass was grown in plastic trays and 100% wheatgrass juice (WJ) was extracted on the 9th day by grinding 3gm of wheatgrass in 2-3ml of water in the electric mixture and then adjusting the final volume to 10ml after sieving the mixture. The different doses of commercial formulation of chlorantraniliprole (CTPR) (18.5% SC) i.e. 1/2 LD_{50} (2500 mg/kg of b.w.), 1/4 LD_{50} (1250 mg/kg of b.w.), 1/6 LD_{50} (833 mg/kg of b.w.), arsenic (As) in the form of sodium arsenite @ 0.50mg/L in drinking water and wheatgrass juice @10ml/kg b.w. were provided to rats orally. The doses of chlorantraniliprole, arsenic and wheatgrass juice were considered based on earlier reported studies (Ghoneim et al., 2018; Mobdy et al., 2017; Moreno et al., 2016). The rats were treated for 28 days and divided into 8 groups with 6 rats in each group as: Group I: 1/2 LD_{50} chlorantraniliprole + arsenic; Group II: 1/4 LD_{50} chlorantraniliprole + arsenic; Group III: 1/6 LD_{50} chlorantraniliprole + arsenic; Group IV:Arsenic; Group V:1/2 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice; Group VI :1/4 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice; Group VII:1/6 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice, Group VIII: Control.

2.3. Estrous Cyclicity

Three consecutive estrous cycles were monitored prior to treatment to confirm rats were cyclic. Throughout the treatment period, the four stages of the estrous cycle were identified by the vaginal smear cytology on the basis of cellular morphology such as the predominance of large nucleated epithelial cells in the proestrus stage, flattened anucleated cornified epithelial cells in the estrus stage, presence of both cornified epithelial cells and leukocytes in the metestrus phase and the abundance of small, round leukocytes in the diestrus stage. Consequently, the total duration of the estrous cycle was recorded.

2.4. Sacrifice of Animals

After the treatment, the rats were subjected to cervical dislocation and blood samples were collected directly from the ventricle by using syringes with heparin to prevent blood clotting. The animals were dissected and the reproductive organs i.e. ovary and uterus were excised, the adhering fat was cleaned off from the organs and these organs were preserved for further histological and biochemical analysis.

2.5. Homogenate Preparation

The tissue (ovary and uterus) were homogenized in a 50 mM phosphate buffer, 2ml (pH 7.4), and the tissue homogenate was centrifuged at 5000 r.p.m. for 20 minutes. The supernatant was collected and used for further biochemical analysis.

2.6. Evaluation of Histological Studies

The ovaries and uterus of the albino rats were cleared from the adhering tissue and fats and were thereby fixed in the Bouin's fixative for 24 hours. After fixing the organs in the Bouin's fixative, the tissues were processed for paraffin block preparation by acetone benzene schedule (Luna, 1968). Sections of approximate thickness of 5-6 μm were cut serially using a rotary microtome**.** Dewaxing and rehydration in descending series of ethanol to water followed with and stained with haematoxylin and counterstained with eosin. The microphotographs were analysed using Magvision software. The number of primordial, primary, secondary, pre-antral, antral as well as corpora lutea were observed in the ovarian sections (Pederson 1970). The percentage of atresia was calculated as follows (Eq.1):

$$\frac{\text{Number of atretic follicles in a stage}}{\text{Number of atretic + healthy follicles of stage}} \times 100$$

The uterine sections were observed for height of uterine epithelium, thickness of myometrium and endometrium, changes in the diameter of endometrial lumen and number of endometrial glands.

2.7. Biochemical Parameters

The biochemical parameters were analysed in the tissue homogenates of ovaries and uterus by the standard methods - Proteins (Lowry et al., 1951), Lipids (Folch et al., 1957), Phospholipids (Ames, 1966), Cholesterol (Zaltkins and Zak., 1968) and Free Fatty Acids (Lowry and Tansley., 1976).

2.8. Statistical Analysis

One-way analysis of variance was done by using Tukey's method in SPSS version 16.0 to compare mean differences among multiple groups, as this approach is appropriate for detecting intergroup differences while controlling Type I error (Tukey, 1949).

3. RESULTS AND DISCUSSION

3.1. Effect on the Estrous Cyclicity

Estrous cyclicity when studied was found to be disturbed in all of the chlorantraniliprole and arsenic treated rats. The control rats however showed normal cyclicity with all four phases of the cycle. The effect was more pronounced in ($1/2LD_{50}$ and $1/4LD_{50}$ chlorantraniliprole + As) treated rats which demonstrated a significant decrease in the number of estrous cycles as compared to control rats and only arsenic treated rats. Rats treated with ($1/6LD_{50}$ chlorantraniliprole + As) showed significant decrease in the number of estrous cycles as compared to control, however this result was non-significant as compared to only As treated rats. Wheatgrass however, showed protective effect as the number of estrous cycles varied non significantly from control in rats administered with wheatgrass (Table 1).

The duration of proestrus and estrus phase in the ($1/2LD_{50}$ and $1/4LD_{50}$ chlorantraniliprole + As) treated rats was found to have significantly reduced as compared to control and only As treated rats, however rats treated with ($1/6LD_{50}$ chlorantraniliprole + As) showed significant decrease in duration of proestrus and estrus phase as compared to control but varied non-significantly from only As treated rats which effectively reversed in rats treated with wheatgrass. The duration of metaestrus and diestrus stage observed in ($1/2LD_{50}$ and $1/4LD_{50}$ chlorantraniliprole + As) treated rats was significantly high as compared to control. As treated rats and rats treated with ($1/6LD_{50}$ chlorantraniliprole + As) showed significant decrease in duration of metaestrus and diestrus phase as compared to control but varied non-significantly from only As treated rats whereas the duration of metaestrus and diestrus stage in rats treated with wheatgrass showed non-significant difference as compared to control which suggested wheatgrass was an effective ameliorative agent (Table 1). These alterations resulted in a significant increase in the total duration of estrous cycle in the ($1/2LD_{50}$ chlorantraniliprole + As) treated rats as compared to control and only arsenic treated rats. The ($1/4LD_{50}$ and $1/6LD_{50}$ chlorantraniliprole + As) treated rats also significant increase in the total duration of estrous cycle as compared to control rats but varied non significantly with only As treated rats. Wheatgrass however, was able to potentially ameliorate the induced toxicity (Table 1). Sharma et al. (2020) investigated the reproductive toxicity caused by carbamates and organophosphate insecticides in female rats and reported significant disruption of ovarian function. Their findings demonstrated impaired follicular development and inhibition of ovulation, leading to alterations in the normal estrous cycle pattern. Prolongation of the diestrus phase was particularly evident, indicating endocrine imbalance and compromised reproductive cyclicity following insecticide exposure. Similarly, Omar et al. (2022) also found that rats treated with coragen showed significant changes in the duration of estrous cycle and a reduced estrus phase. Exposure to coragen as reported by Ponepal et al. (2023) significantly disturbed the estrus cyclicity is wistar rats by prolonging the duration of the diestrus stage which was also observed in the current study. Sprague Dawley rats when exposed to sodium arsenite showed prolonged oestrus cycle and halted diestrus phase owing to disruption in the level of estrogen and progesterone (Mir et al., 2023). Flavanols and phenols alter the intracellular signalling pathways of inflammation and apoptosis, thereby suppressing the pro-inflammatory gene Bcl expression thus inhibiting the apoptotic activities (Panda et al., 2021). As these constituents are also present in wheatgrass, thereby wheatgrass may also be able to mitigate the estrus cyclicity disturbances caused by combined action of chlorantraniliprole and arsenic.

3.2. Histological Changes

The light microscopic studies of the ovary showed various alterations in different group of rats (Table 2, Figure 1 A-H). The significant decreased number of follicles (primary, secondary, tertiary, pre-antral and antral), reduction in the diameter of the follicles and increased number of atretic follicles was observed in chlorantraniliprole (1/2LD_{50} and 1/4LD_{50}) + As treated rats (Figure. B and C) as compared to control (Figure. A) and only Arsenic treated rats (Figure E).

The rats treated with chlorantraniliprole (1/6LD_{50}) + As showed significantly decreased number of normal follicles, decreased follicular diameter and significantly increased number of atretic follicles (Figure D) as compared to control but varied non-significantly from only As treated rats. The increase in the number of normal follicles, decrease in the number of atretic follicles along with increased diameter of follicles was observed in rats treated with chlorantraniliprole (1/2LD_{50}, 1/4LD_{50}, 1/6LD_{50}) + As + wheatgrass as compared to only chlorantraniliprole + As treated rats with effective amelioration by wheatgrass in Chlorantraniliprole (1/6LD_{50}) + As + wheatgrass treated rats. The alterations such as necrosis and vacuolation in the interstitium of the ovarian tissue were observed in the chlorantraniliprole (1/2LD_{50}, 1/4LD_{50}, 1/6LD_{50}) + As treated rats and only arsenic treated rats. However, no such damage of the ovarian tissue was observed in rats administered with wheatgrass along with chlorantraniliprole (1/2LD_{50}, 1/4LD_{50}, 1/6LD_{50}) + As. The histological changes in the ovary such as reduced follicular number and diameter due to the combined effect of coragen and arsenic coincide with the previous studies wherein the female rats exposed to coragen also showed reduced number of follicles with arrested growth in the pre-antral stage,

Table 1. Effect on estrous cyclicity in albino rats exposed to chlorantraniliprole and arsenic along with wheatgrass

Groups	Number of cycles (28 days)	Time duration of one estrous cycle (in days)				
		Proestrus	Estrus	Metaestrus	Diestrus	Total duration of estrous cycle
1/2 LD_{50} chlorantraniliprole + arsenic	4.57^{d}	0.17^{c}	1.21^{d}	0.97^{e}	2.68^{c}	5.03^{c}
1/4 LD_{50} chlorantraniliprole + arsenic	4.75^{d}	0.19^{bc}	1.24^{d}	0.94^{de}	2.62^{c}	4.99^{b}
1/6 LD_{50} chlorantraniliprole + arsenic	5.17^{c}	0.28^{abc}	1.27^{c}	0.89^{cd}	2.24^{b}	4.97^{ab}
Arsenic	5.14^{c}	0.24^{abc}	1.27^{c}	0.89^{cd}	2.17^{b}	4.97^{ab}
1/2 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	5.45^{b}	0.29^{ab}	1.29^{bc}	0.80^{abc}	1.76^{a}	4.98^{ab}
1/4 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	5.59^{ab}	0.32^{ab}	1.31^{ab}	0.78^{ab}	1.62^{a}	4.96^{a}
1/6 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	5.62^{ab}	0.37^{a}	1.33^{a}	0.75^{a}	1.59^{a}	4.97^{a}
Control	5.69^{a}	0.39^{a}	1.36^{a}	0.72^{a}	1.50^{a}	4.97^{a}

Values are expressed as mean values
Different superscript (a-f) in a column indicate significant difference (p≤0.05)

deposits of collagenous tissues in the corpora lutea. Oxidative stress increases the number of atretic follicles in the ovarian cortex along with disorganising the cellular arrangement in the stromal cells, causing loss of demarcation between the epithelial and stromal tissues in the uterus (El-Din et al., 2023). Hamdani and Yajurvedi (2017) also reported increase in the number of atretic follicles in the cortex area covered by surface epithelium due to exposure to cypermethrin. Xiao and Lai (2025) analysed the effect of oxidative stress on the histology of the ovaries induced by heavy metals and concluded the presence of widespread ovarian follicle atresia. Wu et al. (2025) observed the histological changes in the ovarian tissues of female wistar rats exposed to As and showed reduced number of follicles with arrested growth in the preantral stage which were in accordance with the findings of the current study.

The histological slides of uterus revealed the alterations in different group of rats (Table 3, Figure. 2 A-H). chlorantraniliprole (1/2LD_{50} and 1/4LD_{50}) + As treated rats showed decreased uterine luminal diameter, endometrial and myometrial thickness and number of uterine glands (Figure. 2B, 2C) as compared to control (Figure. 2A, Table 3) and only arsenic exposed rats (Figure. 2E, Table 3). However, rats treated with chlorantraniliprole (1/6LD_{50}) + As showed significant decrease in luminal diameter, endometrial and myometrial thickness and number of uterine glands (Figure. 2D) as compared to control but varied non-significantly from only As treated rats. The rats treated with chlorantraniliprole (1/2LD_{50}, 1/4LD_{50}, 1/6LD_{50})+ As + wheatgrass showed significant recovery in the uterine luminal diameter, endometrial and myometrial thickness and number of uterine glands (Figure. 2F, 2G, 2H) as compared to only chlorantraniliprole + As treated rats with effective amelioration by wheatgrass against the combined toxicity due to chlorantraniliprole (1/6LD_{50}) and arsenic. Hassan et al. (2021) also reported that exposure to coragen caused accumulation of fibrous connective tissues in the stroma of uterus, follicles were also surrounded by pyknotic granulosa cells arranged in the form of symmetric rings below the basement membrane. Erthal et al. (2024) observed

Table 2. Effect on number and diameter of ovarian follicles in albino rats exposed to chlorantraniliprole and arsenic along with wheatgrass

Groups	Number of ovarian follicles						Diameter of ovarian follicles			
	Primordial follicle	Primary follicle	Secondary follicle	Pre-antral follicle	Antral follicle	Atretic Follicle	Primary follicle	Secondary follicle	Pre-antral follicle	Antral follicle
1/2 LD_{50} chlorantraniliprole + arsenic	5.33^{f}	0.33^{e}	0.33^{c}	0.33^{c}	0.33^{c}	3.66^{c}	31.37^{d}	76.38^{d}	152.70^{f}	217.21^{e}
1/4 LD_{50} chlorantraniliprole + arsenic	7.33^{e}	1.66^{de}	0.66^{c}	0.33^{c}	0.33^{c}	3.33^{c}	34.19^{d}	80.17^{d}	174.27^{e}	231.24^{d}
1/6 LD_{50} chlorantraniliprole + arsenic	10.33^{d}	2.66^{cd}	1.66^{ab}	0.66^{b}	0.66^{b}	2.33^{b}	38.93^{c}	85.39^{c}	196.48^{d}	256.27^{c}
Arsenic	9.33^{d}	2.00^{bcd}	1.33^{ab}	0.63^{b}	0.66^{b}	2.60^{bc}	38.62^{c}	85.22^{c}	196.16^{d}	257.30^{c}
1/2 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	12.66^{c}	3.33^{abc}	2.00^{a}	1.20^{a}	1.00^{a}	1.33^{ab}	42.11^{b}	90.36^{b}	212.87^{c}	275.29^{b}
1/4 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	16.33^{b}	4.00^{ab}	2.33^{a}	1.36^{a}	1.25^{a}	1.00^{ab}	46.77^{ab}	94.19^{ab}	236.97^{b}	296.31^{ab}
1/6 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	17.66^{ab}	4.33^{a}	2.66^{a}	1.60^{a}	1.30^{a}	0.66^{a}	49.87^{a}	96.03^{a}	249.02^{a}	307.33^{a}
Control	19.00^{a}	4.66^{a}	2.66^{a}	1.66^{a}	1.33^{a}	0.66^{a}	50.23^{a}	97.45^{a}	257.11^{a}	309.89^{a}

See Table 1 for details

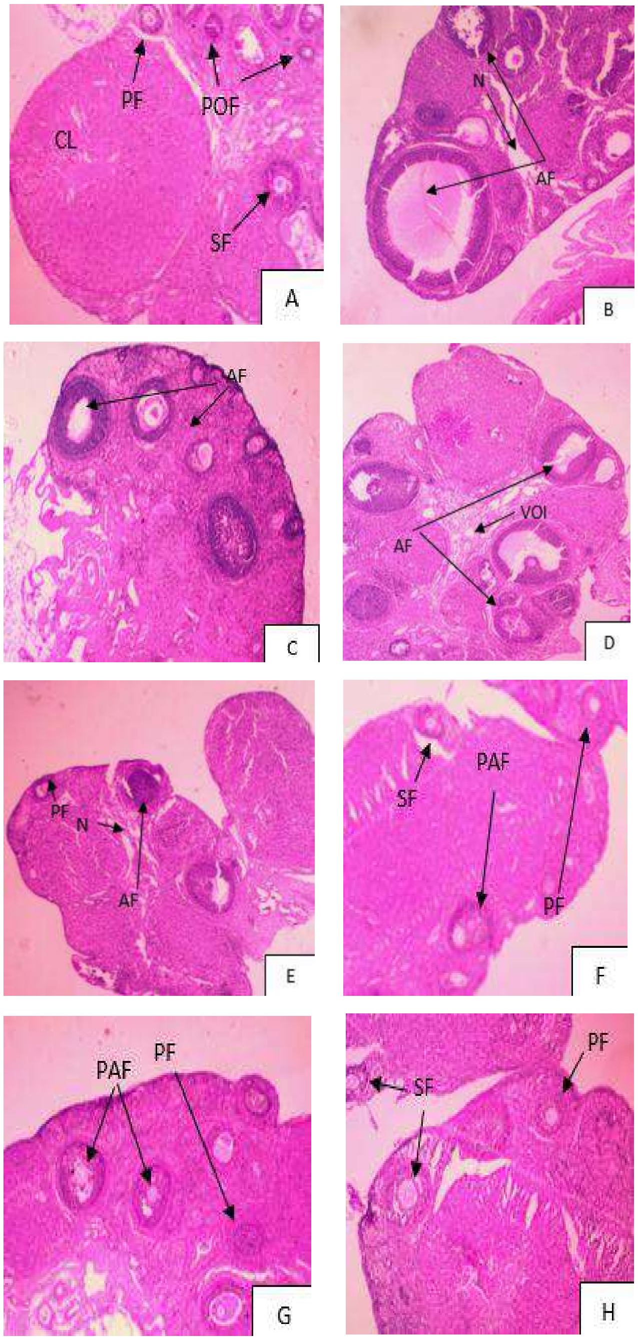

Figure 1. A-H: T.S. of ovary (X100) A Control B 1/2 LD_{50} chlorantraniliprole + arsenic C 1/4 LD_{50} chlorantraniliprole + arsenic D 1/6 LD_{50} chlorantraniliprole + arsenic E Arsenic F 1/2 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice G 1/4 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice H 1/6 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice (PF-primary follicle, SF- secondary follicle, CL-corpus luteum, AF- atretic follicle, N-necrosis, V-vacuolisation, PAF-primary atretic follicle)

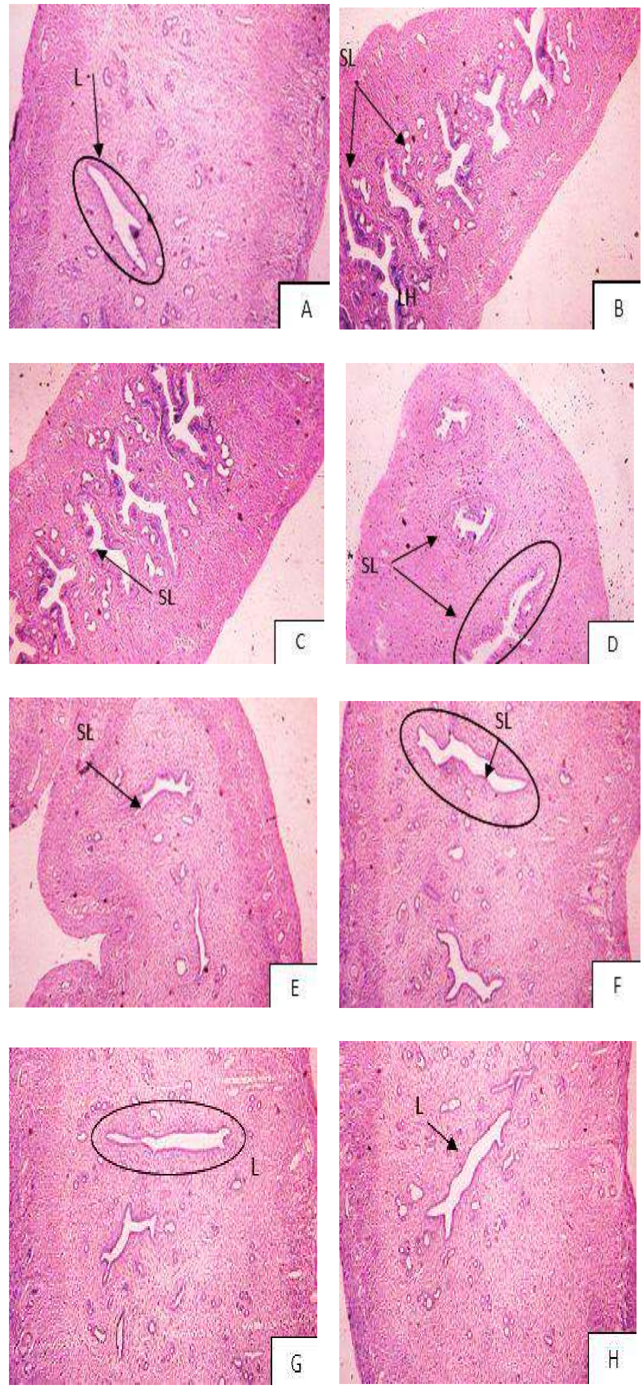

Figure 2. A-H: T.S. of uterus (X100) A Control B 1/2 LD_{50} chlorantraniliprole + arsenic C 1/4 LD_{50} chlorantraniliprole + arsenic D 1/6 LD_{50} chlorantraniliprole + arsenic E Arsenic F 1/2 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice G 1/4 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice H 1/6 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice (L- Normal Lumen, SL- Scattered lumen)

that exposure of malathion in albino rats showed squamous hyperplasia and cornified vaginal and epithelium in the uterus along with endometrial and myometrial edema. Barbhuiya et al. (2021) studied that arsenic induced reproductive toxicity in female albino rats and observed that epithelial and myometrial diameter of uterus showed significant decrease along with a narrow and unfolded lumen. Methomyl exposure leads to reduced myometrial and endometrial diameter along with causing apoptosis in the uterine cells as suggested by Mosbah et al. (2024) which were also seen in the current study. Since wheatgrass is rich in active antioxidants it can potentially reduce the oxidative damage resulting in histological alterations and thereby showing the recovery which has been observed.

3.3. Biochemical Parameters

Biochemical parameters (Table 4) in the ovaries and uterus revealed significant reduction in the content of total soluble protein, total lipids, phospholipids and free fatty acids in chlorantraniliprole ($1/2LD_{50}$ and $1/4LD_{50}$) + As treated rats as compared to control and only arsenic treated rats whereas, rats treated with chlorantraniliprole ($1/6LD_{50}$) + As showed significant decrease in content of total soluble proteins, total lipids, phospholipids, free fatty acids as compared to control but varied non-significantly from only As treated rats. However, rats treated with chlorantraniliprole + As + wheatgrass as compared to only

Table 3. Effect on uterine parameters in the albino rats exposed to chlorantraniliprole and arsenic along with wheatgrass

Groups	Diameter of uterine lumen (µm)	Endometrial thickness (µm)	Myometrial thickness (µm)
1/2 LD_{50} chlorantraniliprole + arsenic	42.57^{g}	261.60^{e}	53.99^{a}
1/4 LD_{50} chlorantraniliprole + arsenic	52.57^{f}	300.78^{d}	65.40^{e}
1/6 LD_{50} chlorantraniliprole + arsenic	58.39^{e}	342.17^{c}	82.99^{d}
Arsenic	57.17^{d}	339.93^{c}	85.06^{d}
1/2 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	60.92^{c}	372.59^{b}	104.59^{c}
1/4 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	64.30^{b}	404.62^{b}	142.83^{b}
1/6 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	69.40^{a}	419.31^{a}	161.57^{a}
Control	70.85^{a}	426.80^{a}	172.47^{a}

See Table 1 for details

Table 4. Effect on the biochemical parameters in the reproductive organs of albino rats exposed to chlorantraniliprole and arsenic along with wheatgrass

Groups	Total soluble protein (mg/g of tissue)		Total lipids (mg/g of tissue)		Phospholipids (mg/g of tissue)		Free fatty acid (mg/g of tissue)		Cholesterol (mg/g of tissue)	
	Ovary	Uterus	Ovary	Uterus	Ovary	Uterus	Ovary	Uterus	Ovary	Uterus
1/2 LD_{50} chlorantraniliprole + arsenic	1.24^{f}	3.59^{e}	46.66^{c}	71.66^{c}	7.38^{d}	10.21^{f}	0.67^{e}	1.01^{e}	2.38^{d}	4.21^{f}
1/4 LD_{50} chlorantraniliprole + arsenic	1.96^{e}	4.35^{d}	49.33^{c}	75.33^{c}	8.31^{d}	14.63^{e}	0.81^{d}	1.31^{d}	1.91^{c}	3.62^{e}
1/6 LD_{50} chlorantraniliprole + arsenic	2.58^{d}	5.53^{c}	55.67^{b}	78.66^{b}	9.58^{c}	18.24^{d}	0.92^{c}	1.74^{c}	1.63^{bcd}	3.13^{d}
Arsenic	2.54^{d}	5.23^{c}	53.66^{b}	78.33^{b}	9.65^{c}	18.43^{d}	0.95^{c}	1.63^{c}	1.71^{cd}	3.23^{d}
1/2 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	2.77^{bc}	6.71^{b}	55.31^{a}	81.66^{a}	14.49^{b}	22.5^{c}	1.15^{b}	2.20^{b}	1.58^{abc}	2.72^{c}
1/4 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	2.95^{bc}	8.76^{b}	56.23^{a}	82.66^{a}	15.23^{ab}	24.67^{ab}	1.37^{a}	2.39^{ab}	1.47^{ab}	2.49^{ab}
1/6 LD_{50} chlorantraniliprole + arsenic + wheatgrass juice	3.10^{ab}	9.09^{a}	57.06^{a}	83.21^{a}	15.39^{ab}	24.75^{ab}	1.41^{a}	2.58^{a}	1.41^{ab}	2.33^{a}
Control	3.27^{a}	9.54^{a}	58.69^{a}	84.3^{a}	15.44^{a}	26.28^{a}	1.46^{a}	2.56^{a}	1.36^{a}	2.26^{a}

See Table 1 for details

chlorantraniliprole + As treated rats suggested the ability of wheatgrass to protect against the combined toxicity of chlorantraniliprole and arsenic with maximum effect in chlorantraniliprole (1/4LD_{50} and 1/6LD_{50}) + As + wheatgrass treated rats. Similar observations were reported due to combined effect of arsenic and chlorpyirfos that resulted in decreased content of proteins as the ROS bind to thiol groups of amino acids and disrupt their macromolecular structures thus increasing the proteasomal degradation of proteins (Jasbeen and Noor, 2024). The decrease in the level of lipids and phospholipids observed was because of the polyunsaturated fatty acids (PUFAs) that are present in the cell membranes, are extremely sensitive to ROS as they modify the lipid composition, structure and dynamics of cell membranes because of lipid peroxidation and thereby decrease the content of total lipids and phospholipids that was observed by the combined effect of arsenic, cadmium and mercury (Nie et al., 2024). Shivyari et al. (2024) suggested that the level of free fatty acids decreases under oxidative stress as the triglycerides are taken up by the cells in the form of free fatty acids and further result in decreased polyunsaturated fatty acid among total free fatty acid content. Wheatgrass extracts also have flavonoid and phenolic content that inhibit ascorbate-Fe^{2+} induced lipid peroxidation in the liver and mitochondria of rats (Aldaamy et al., 2023). These changes are suggestive of why wheatgrass was able to effectively ameliorate the lipid peroxidation levels in the current study.

The content of cholesterol in the ovarian and uterine tissue in chlorantraniliprole (1/2LD_{50} and 1/4LD_{50}) + As treated rats showed significant increase as compared to control and only arsenic treated rats, whereas rats treated with chlorantraniliprole (1/6LD_{50}) + As showed significant increase in content of cholesterol as compared to control but varied non-significantly from only As treated rats. The groups administered with chlorantraniliprole + As + wheatgrass however, were able to mitigate the toxic effects of chlorantraniliprole and arsenic. The increased level of cholesterol reported in the present study is due to accumulation of cholesterol in the form of esters because of deficiency of the enzyme lecithin-cholesterol acyltransferase from the liver (Zhang et al., 2024).

4. CONCLUSION

The combination of variable doses of chlorantraniliprole and arsenic caused reproductive toxicity in albino rats by inducing the production of reactive oxygen species (ROS) which caused an imbalance in the functioning of enzymes involved in regulating the oxidative stress and thereby disrupt the other physiological functions of the reproductive system. Wheatgrass owing to its antioxidant properties is capable of balancing the increased oxidative stress and restoring the compromised reproductive functions caused by these prevalent environmental toxicants thereby depicting the role of wheatgrass as a promising ameliorative agent that can be explored for developing plant-based nutraceuticals or adjunct therapeutic formulations aimed at mitigating oxidative stress induced reproductive dysfunction caused by environmental toxicants.

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Authors' Contributions

SB: Data curation, Formal analysis, Investigation, Methodology, Writing the original draft, Writing- reviewing and editing. NK: Conceptualisation, Project administration, Supervision, Writing- reviewing and editing.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Generative AI and AI-assisted technologies in the writing process

No use of AI tools have been used in the writing process.

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