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EVALUATION OF PROTECTIVE EFFICACY OF *PUNICA GRANATUM* ON SHORT TERM β-CYFLUTHRIN TOXICITY ON REPRODUCTIVE TISSUE OF MALE MICE

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ABSTRACT: Pyrethroids are among the most extensively used insecticide. The widespread use of these synthetic pesticides has stimulated research into the possible existence of adverse effects related to reproductive activity. The present study was, therefore, undertaken to assess the effects of β- Cyfluthrin, a type II synthetic pyrethroid on testes, the main organ of male reproduction. Animals were divided into five groups- Group I: Control, Group II: Vehicle control (corn oil), Group III: β-Cyfluthrin treated, Group IV: *Punica granatum* juice, and Group V: Pesticide + Antidote (β-Cyfluthrin + Antidote) treated. β-Cyfluthrin and *Punica granatum* juice both were administered orally to male albino mice of Swiss strain for 21 days to evaluate the toxic alterations in gravimetric and biochemical parameters. The body weight as well as testicular weight of animals showed significant reduction after β-Cyfluthrin treatment. Also, biochemical parameters like protein content, ATPase, SDH, and ALPase activity were seen to reduce significantly. Whereas, ACPase activity was observed to increase in β-Cyfluthrin administered group. In contrast, administration of β-Cyfluthrin and PJ concomitantly showed a protective effect of PJ against β-Cyfluthrin toxicity by improving various biochemical alterations. Therefore, from the results of the present study, it can be concluded that β-Cyfluthrin induces severe testicular damage and thus can affect fertility which can be attenuated by intake of PJ to a great extent. Therefore, it is recommended that people getting exposed to pesticide continuously incorporate PJ on a daily basis in the diet.

INTRODUCTION: Humans get exposed to an enormous number of possible mixtures of chemicals via different routes in their day-to-day life [1]. It has been reported that pesticides followed by pharmaceuticals and personal care products dominate the observed mixture effects in the environment [2], and the occurrence of potential combination effects of pesticides is an area of increasing concern for the public and regulatory authorities.

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Extensive use of pesticides all over the world greatly promote the agricultural development, but since use of this pesticides is indiscriminant in agriculture, the pesticide residuals in food, water and soil pose a significant threat to human health [3, 4].

Further, four major classes of chemical insecticides, namely organochlorines (exclusively DDT), organophosphates, carbamates, and pyrethroids are the mainstay of vector control programs, among which pyrethroids are considered as the most successful class, providing high potency against a wide variety of arthropods. Therefore, these synthetic pyrethroids now constitute the majority of commercial insecticides and account for approximately 25% of the global

insecticide market [5, 6]. Despite being considered relatively safe for humans; epidemiological data, clinical reports, and laboratory studies indicate that pyrethroid exposure leads to neurotoxic and immunotoxic effects in humans and animals [7]. β-cyfluthrin is one such pyrethroid which is the refined form of the synthetic pyrethroid cyfluthrin and is currently being used in many formulations worldwide [8]. It is a type II fluorinated pyrethroid pesticide which has broad potential against the target as well as non-target species [9, 10] and widely used in agriculture and other domestic application [11]. Ansari *et al.* (2012) [12] reported behavioural deficits associated with pyrethroid exposure along with impairment of motor activity, grip strength, learning ability. The neurotoxic effects of β-cyfluthrin and other pyrethroids are primarily mediated through their interaction with sodium channels, leading to depolarization and hyper-excitation of the nervous system. Many studies have also shown that excessive exposure to pyrethroids could induce damage to the liver, and the degree of damage is associated with the dose and the duration of exposure [13, 14]. Therefore, injury to such vital organs of the body poses serious medical problems which must be properly managed.

On the basis of easy availability, abundance, and economic, plants have been the basic source of therapeutic agents used more frequently by human resources. In spite of the lifestyle changes of people on a regular basis, there are a large number of tribal communities who are still utilizing the plant genetic resources as medicine occurring in their surrounding vegetation [15]. One of such traditionally used plants is *Punica granatum*.

Pomegranate (*Punica granatum* Linn.) belongs to the Punicaceae family, widely cultivated in the Mediterranean region. Its fruit is a well-known source of bioactive compounds and has been used since ancient times and for centuries in folk medicine. Antioxidants in *P. granatum* are from polyphenolic class that includes tannins, anthocynins, flavonoids, punicalagin, ellagic, and gallic acid [16, 17]. Polyphenols are a large group of plants, which have high antioxidant activity [18, 19]. Traditional use has received attention by the scientific community; the *in-vitro* and *in-vivo* studies carried out recently have demonstrated its antioxidant [20, 21, 22], anticancer [23], anti-inflammatory [24, 25], anti-hyperlipidemic [26], photo-protector [21], antiviral [27], antimicrobial [28], and antifungal [29] properties. Moreover, *Punica granatum* has beneficial properties on spermato-genesis [30]. Also, it can be used in the prevention and treatment of cardiovascular disease, osteoarthritis, rheumatoid arthritis, and other diseases [31]. Shaoul *et al.* (2018) [32] also evaluated the protective effects of *Punica granatum* oral supplementation on intestinal structural changes, enterocyte proliferation, and apoptosis during methotrexate (MTX)-induced intestinal damage in a rat. *P. granatum* also has antibacterial properties and has the therapeutic potential for prostate cancer, erectile dysfunction, Alzheimer's, and also reducing obesity since they have both ellagic acid and punicalagin content in the fruit [33, 34].

The therapeutic and medicinal value of *Punica granatum* is the subject of many researchers. To our knowledge, the potential protective effect of pomegranate (*Punica granatum*) juice on β-cyfluthrin induced reproductive toxicity in male mice has not been explored. In view of these considerations, the functional health benefits of *Punica granatum* in protecting reproductive tissues against pyrethroid insecticide would be of current interest. Therefore, the aim of the study was to evaluate the protective efficacy of *Punica granatum* juice on β-cyfluthrin induced biochemical alteration in reproductive tissues of Swiss albino mice.

MATERIALS AND METHODS:

Animals: Healthy, adult, pathogen-free, colony-bred male albino mice (*Mus musculus*) of Swiss strain weighing between 30-40 g obtained from recognized supplier Cadila Pharmaceuticals, Dholka, Gujarat, India. The experimental protocol and the number of animals used for the experiments were mentioned in a detailed proposal, and approval was obtained as per the guidelines of the institutional animal ethics committee, under registration No. 167/GO/ReBi/S/99/CPCSEA from the Ministry of Social Justice and Empowerment, Government of India and Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India. All the animals were acclimatized for seven days prior to the commencement of the experiment. The animals

were housed in an air-conditioned animal house at a temperature of 26 ± 2 ºC and exposed to 10 –12 hr of daylight and relative humidity of 40%–50%. Animals were randomized into control and treated groups and were caged separately. Standard chow (obtained from Amrut laboratory, Baroda, India) and water were provided *ad libitum*.

Chemicals: Technical grade β-cyfluthrin of 95% purity was procured from Nanjing Essence Fine chemicals, China. All the other chemicals used in different assays were procured from HiMedia or Merck.

Experimental Design: β-cyfluthrin was dissolved in Corn oil and administered via oral gavage at a dose of 13 mg/kg body weight ($1/20^{th}$ of LD_{50}). The dose was determined on the basis of LD_{50} value of β-cyfluthrin in corn oil, i.e., 260 mg/kg body weight [35]. Animals were divided into the following groups (6 animals per group).

Group I: Control (given water and food *ad libitum*);

Group II: Vehicle Control (corn oil);

Group III: β -cyfluthrin treated (given 13 mg/kg body weight β-cyfluthrin dissolved in corn oil);

Group IV: *Punica granatum* Juice;

Group V: β-cyfluthrin + *Punica granatum* juice.

All the groups were treated for 21 days, and at the end of the experiment, animals were weighed and euthanized using light ether anesthesia. Animals were dissected, and tissue, namely, Testis was dissected out. Tissue was weighed, and homo-genates were prepared accordingly.

Preparation of *Punica granatum* Juice (PJ): Fresh ripe red pomegranate fruit was purchased from the local market in Ahmedabad. The fruit was properly washed and manually peeled without separating the seeds. The juice was obtained using a commercial blender and was then filtered. The dose preparation of pomegranate juice was based on the studies of Turk *et al*. (2008) [30]. Fresh 0.2 ml of PJ was given every day by oral intubation for the entire experimental duration of 21 days.

Parameters Studied:

Body and Organ Weights: The body weight of control and all treated groups of mice were recorded daily to the nearest gram on a digital balance (Reptech). Similarly, weights of organs were recorded after euthanizing to the nearest milligram on digital balance (Aczet CY 224C).

Total Proteins: Protein levels in the testis of control and other treated groups of animals were estimated by the method of Lowry *et al*., (1951) [36]. The sample containing protein was treated with phenol reagent of Folin Ciocalteau, a deep blue colour developed due to two reactions occurring simultaneously, *i.e.* the reaction of alkaline copper sulphate solution with peptide bonds and the reduction of phosphomolybdic and phosphotungstic acids by aromatic amino acids present in the protein. The blue colour developed is quantitatively proportional to the total proteins, which is measured on Lab India UV/VIS 3000 + Spectrophotometer at 540 nm and expressed as mg/dL.

Succinate Dehydrogenase (SDH): SDH activity was measured by the method of Beatty et al. (1966) [37]. The electrons released by the enzyme SDH from the substrate are taken up by an electron acceptor INT which is reduced to red coloured formazan. After extracting it in ethylacetate the colour intensity was measured at 420 nm against a blank. SDH activity was expressed as µg formazan formed/15 min/mg tissue weight.

Adenosine Triphosphatase (ATPase): The ATPase activity in testis of control and all treated groups of animals was assayed by the method of Quinn and White (1968) [38]; while inorganic phosphate liberated was estimated using the method of Fiske and Subbarow (1925) [39]. Readings were taken at 660 nm on a Lab India UV/VIS 3000+ Spectrophotometer.

Alkaline Phosphatase (ALPase): Alkaline Phos-phatase (ALPase) activity was determined by the method of Bessey *et al. (*1946) [40]. The enzyme ALPase hydrolyses the substrate p-nitro phenyl phosphate into inorganic phosphate and p-nitrophenol. The quantity of p-nitrophenol released under standardized condition was measured at 410 nm. Enzyme activity was expressed as µ moles p-nitrophenol released/30 minutes/mg protein.

Acid Phosphatase (ACPase): The activity of ACPase was determined by the method of Bessey

et al. (1946) [40]. ACPase catalyzes the hydrolysis of p-nitrophenol nitrate at pH 4.8, liberating p-nitrophenol and inorganic phosphate. The liberated p-nitrophenol combines with NaOH to form a yellow-colored complex which is measured at 420 nm and is directly proportional to the enzyme activity. Enzyme activity was expressed as µ moles of p-nitrophenol released/30 min/mg protein.

Statistical Analysis: For each parameter, a minimum of 6 replicates were done, and the results were expressed as Mean ± Standard Error (S.E.). The data was then statistically analyzed by Analysis of Variance (One way - ANOVA) by Graph-pad Prism software version 8.0.1, taking significance at $p<0.05$. Vehicle control, β-cyfluthrin treated, and *Punica granatum* juice administered groups were compared with control group. β-cyfluthrin + *Punica granatum* juice was compared with β-cyfluthrin treated group.

RESULTS:

Gravimetric Indices:

Body Weight: Administration of β-cyfluthrin (Group III) for 21 days recorded a significant ($p<0.001$) decline in the body weight of experimental model Swiss albino male mice.

Further, Vehicle control and PJ administered (Group II and IV, respectively) showed insignificant results when compared to Group I (control). Supplementation of β-cyfluthrin with *Punica granatum* juice (Group V) witnessed a significant ($p<0.033$) increase in body weight when compared to Group III **Table 1**.

TABLE 1: BODY WEIGHT OF CONTROL AND TREATED ANIMALS FOR DURATION OF 21 DAYS

	Groups	Body Weight (Grams)
I	Control	35.03 ± 0.43
II	Vehicle control (corn oil)	34.57 ± 0.59^{ns}
III	β-cyfluthrin treated	29.87 ± 0.49***
IV	*Punica granatum* juice	34.58 ± 0.70^{ns}
V	β-cyfluthrin + *Punica granatum* juice	$32.3 \pm 0.17^{\#}$

N=6, Values are represented as Mean ± S.E. ***p<0.001, ns - non-significant (compared to control) #p<0.033 (compared to treated group). Analysis of Variance at p<0.05 level.

Organ Weight: Results of 21 days treatment of β-cyfluthrin on organ weight (Testes) revealed a significant ($p<0.001$) decline in Group III, while Group II and IV reported non-significant changes in Testes weight when compared to control group. Group V (β-cyfluthrin + *Punica granatum* juice) recorded a significant ($p<0.002$) increase in Testes weight when compared to Group III (β-cyfluthrin treated) **Table 2**.

TABLE 2: ORGAN WEIGHT (TESTES) OF CONTROL AND TREATED ANIMALS FOR DURATION OF 21 DAYS

	Groups	Organ Weight (mg)
I	Control	279.1 ± 7. 64
II	Vehicle control (corn oil)	270.9 ± 6.51^{ns}
III	β-cyfluthrin treated	203.5 ± 4.02***
IV	*Punica granatum* juice	274 ± 8.97^{ns}
V	β-cyfluthrin + *Punica granatum* juice	$240.1 \pm 5.31^{\#\#}$

N=6, Values are represented as Mean ± S.E. ***p<0.001, ns – non-significant (compared to control) ## p<0.002 (compared to treated group). Analysis of Variance at p<0.05 level.

Biochemical Indices:

Protein Content: Oral administration of β-cyfluthrin for 21 days revealed a significant reduction ($p<0.001$) in protein content in testis tissue in Group III, while Group II and IV showed a non-significant alteration when compared with Group I. Further, supplementation of PJ along with β-cyfluthrin showed a significant ($p<0.033$) rise in protein content in Group V when compared with Group III **Table 3**.

TABLE 3: PROTEIN CONTENT (mg PROTEIN/100 mg FRESH TISSUE WEIGHT) IN TESTIS OF CONTROL AND TREATED ANIMALS FOR DURATION OF 21 DAYS

	Groups	Protein Content (mg Protein/100 mg Fresh Tissue Weight)
I	Control	9.392 ± 0.17
II	Vehicle control (corn oil)	8.878 ± 0.53^{ns}
III	β-cyfluthrin treated	6.468 ± 0.35***
IV	*Punica granatum* juice	8.792 ± 0.32^{ns}
V	β-cyfluthrin + *Punica granatum* juice	$8.13 \pm 0.20^{\#}$

N=6, Values are represented as Mean ± S.E. ***p<0.001, ns – non-significant (compared to control) #p<0.033 (compared to treated group). Analysis of Variance at p<0.05 level.

Succinate Dehydrogenase (SDH): When compared to Group I (control), Group II and IV did not show significant ($p<0.001$) SDH activity after 21 days of treatment duration, while a significant decrease in Group III (β-cyfluthrin treated) was observed in Testis after the administration of said pesticide. Group V (β-cyfluthrin + *Punica granatum* juice), when compared with Group III reported a significant ($p<0.002$) increase in SDH activity **Table 4**.

TABLE 4: ACTIVITY OF SUCCINATE DEHYDROGENASE (µG FORMAZAN FORMED/15 MIN/mg TISSUE WEIGHT) IN TESTIS OF CONTROL AND TREATED ANIMALS FOR DURATION OF 21 DAYS

	Groups	SDH Activity (µg Formazan Formed/15 min/mg Tissue Weight)
I	Control	189.5 ± 3.45
II	Vehicle control (corn oil)	187.8 ± 2.44^{ns}
III	β-cyfluthrin treated	165.8 ± 3.22***
IV	*Punica granatum* juice	199.8 ± 3.53^{ns}
V	*Punica granatum* juice + β-cyfluthrin	182.2 ± 2.53$^{\#\#}$

N=6, Values are represented as Mean ± S.E. ***p<0.001, ns – non-significant (compared to control) ## p<0.002 (compared to treated group). Analysis of Variance at p<0.05 level.

Adenosine Triphosphatase (ATPase): ATPase activity in testis was also seen to significantly (p<0.001) decline after administration of β-cyfluthrin alone in Group III when compared to Group I.

Group II and IV recorded non-significant changes. Compared to Group III, Group V reported a highly significant (p<0.001) increase in ATPase activity after administration of β-cyfluthrin and PJ together **Table 5**.

TABLE 5: ACTIVITY OF ADENOSINE TRIPHOSPHATASE (µ MOLES OF INORGANIC PHOSPHATE (ip) RELEASED/ mg PROTEIN/30 MINUTES) IN TESTIS OF CONTROL AND TREATED ANIMALS FOR DURATION OF 21 DAYS

	Groups	ATPase Activity (µ Moles of Inorganic Phosphate (ip) Released/mg Protein/30 min)
I	Control	1.907 ± 0.033
II	Vehicle control (corn oil)	1.88 ± 0.044^{ns}
III	β-cyfluthrin treated	1.11 ± 0.073***
IV	*Punica granatum* juice	1.865 ± 0.036^{ns}
V	β-cyfluthrin + *Punica granatum* juice	1.567 ± 0.086$^{\#\#\#}$

N=6, Values are represented as Mean ± S.E. ***p<0.001, ns – non-significant (compared to control) ###p<0.001 (compared to treated group). Analysis of Variance at p<0.05 level.

Alkaline Phosphatase (ALPase): When compared to Group 1 (Control), Group II and IV did not reveal significant changes in ALPase activity, while it was observed to decrease significantly (p<0.001) in pesticide-treated (Group III).

Oral supplementation of β-cyfluthrin with *Punica granatum* juice brought the activity of ALPase near to the control value and increased the activity significantly (p<0.002) when compared with Group III (β-cyfluthrin treated) **Table 6.**

TABLE 6: ACTIVITY OF ALKALINE PHOSPHATASE (µ MOLES p-NITROPHENOL RELEASED/30 MIN/mg PROTEIN) IN TESTIS OF CONTROL AND TREATED ANIMALS FOR DURATION OF 21 DAYS

	Groups	ALPase Activity (µ Moles p-nitrophenol Released/30 min/mg Protein)
I	Control	1.653 ± 0.031
II	Vehicle control (corn oil)	1.615 ± 0.016^{ns}
III	β-cyfluthrin treated	1.393 ± 0.051***
IV	*Punica granatum* juice	1.6 ± 0.020^{ns}
V	*Punica granatum* juice + β-cyfluthrin	1.562 ± 0.032$^{\#\#}$

N=6, Values are represented as Mean ± S.E. ***p<0.001, ns – non-significant (compared to control) ## p<0.002 (compared to treated group). Analysis of Variance at p<0.05 level.

Acid phosphatase (ACPase): ACPase activity was seen to rise significantly (p<0.001) in pesticide treated group (Group III), whereas Group II (vehicle control) and Group IV (PJ administered) resulted in non-significant alterations when compared to Control (Group I). Oral administration of Punica granatum juice with β-cyfluthrin (Group V) reduced ACPase activity significantly (p<0.001) when compared with Group III **Table 7.**

TABLE 7: ACTIVITY OF ACID PHOSPHATASE (µ MOLES OF p-NITROPHENOL RELEASED/30 MIN/mg PROTEIN) IN TESTIS OF CONTROL AND TREATED ANIMALS FOR DURATION OF 21 DAYS

	Groups	ACPase Activity (µ Moles of p-nitrophenol Released/30 min/mg Protein)
I	Control	1.727 ± 0.041
II	Vehicle control (corn oil)	1.847 ± 0.029ns
III	β-cyfluthrin treated	2.312 ± 0.036***
IV	*Punica granatum* juice	1.747 ± 0.015^{ns}
V	β-cyfluthrin + *Punica granatum* juice	1.617 ± 0.032$^{\#\#\#}$

N=6, Values are represented as Mean ± S.E. ***p<0.001, ns – non-significant (compared to control) ###p<0.001 (compared to treated group). Analysis of Variance at p<0.05 level

DISCUSSION: The increasing use of chemicals contaminating the environment draws attention to a better understanding of their toxicity in humans and animals [41]. Xenobiotics and environmental contaminants such as pesticides are known to induce a broad spectrum of toxicological effects and biochemical dysfunctions constituting serious hazards to health [42]. Insecticides are chemicals used widely in agriculture, environmental, human, and animal health fields. Exposure to insecticides has been associated with many hazardous effects [43]. Determination of the common mechanism of toxicity in mammals is complicated by the number of potential biological target sites and effects

exerted by various pyrethroid insecticides on these targets [44]. Therefore, the present study was formulated to evaluate certain biochemical parameters on reproductive tissues of Swiss albino male mice after administration of β–cyfluthrin for 21 days. Physiological changes in experimental animals such as body weights and relative organs weights are important criteria for toxicological studies of xenobiotic [45]. A significant decline in body weights of animals treated with β–cyfluthrin was observed when compared to control. Verma et al. [46] and Mohafrash et al. (2017) [45] also reported a significant decline in body weights after β-cyfluthrin administration for 7 and 14 days in albino mice and for 60 days in male albino rats, respectively. Further, Ince *et al.* (2012) [47] also observed a significant decline in body weight of male albino mice of Swiss strain after administration of cypermethrin for 28 days. Reduction in body weight can be attributed to hypophagia and toxic implications of the test substance in experimental animals.

Testis is an important target for endocrine disruption as it functions as both an endocrine gland as well as a reproductive organ, responsible for the production of hormones and male gametes. In the present study, a decrease in the testicular weight was observed, which can be correlated with a decline in protein content after 21 days of toxicant treatment. Rajawat *et al.*, (2014) [48] also revealed reduced testis weight in Swiss albino mice after cyfluthrin treatment. Further, they reported that histopathologically cyfluthrin causes various structural abnormalities in testes, seminiferous tubules were shrunken and appeared to be displaced, lumen diameter was decreased, and vacuolization occurred in the interstitial spaces, which may contribute to reduced testicular weight.

Insecticides interfere with important biochemical and enzymatic processes that regulate the normal physiology of animals. These insecticide mediated biochemical changes can challenge the homeostasis of organism and also affect the normal functioning of their organs which limit the potential of an animal population in effectively cope up with normal stress and survival [49]. Free radicals are among the major etiological factors implicated in several pesticide toxicities [50], which can attack macro-molecules *via* increasing phospholipid, protein, and DNA oxidation [51]. A significant decline in protein content of testis was observed in the present study after β-cyfluthrin treatment for 21 days. A similar decline in total serum protein was observed in fishes exposed to deltamethrin by Vani *et al.* (2011) [52]. Bhushan *et al.* (2013) [53] also reported reduced protein content in the liver of Wistar rats after administration of cypermethrin and β–cyfluthrin during acute as well as sub-acute pesticides toxicity studies. Also, Gupta and Sharma (2016) [54] recorded duration-dependent reduced protein content in liver and muscle tissues of *Channa punctatus* after administration of cypermethrin.

Pyrethroids are not highly cytotoxic [55], but pyrethroids have been shown to interact with many membrane proteins. Adenosine tri-phosphatase (ATPase), a membrane-associated enzyme synthesized in the inner mitochondrial matrix; catalyze the breakdown of Adenosine triphosphate (ATP) into Adenosine di-phosphate (ADP) with a free phosphate ion (dephosphorylation), which ultimately leads to the release of energy [56].

The enzymes Na^+ /K^+ ATPases and Mg^{2+} ATPases have a relatively high sensitivity to certain classes of heavy metals and other pollutants [57]. In the current study, it has been observed that there is a significant reduction in the activity of ATPase, which might result in reduced cation exchange through the membrane and thereby its reduced energy-dependent secretory functions. In support of our results, Kakko *et al.*, (2003) [58] also recorded reduced ATPase activity in male Sprague– Dawley rats after treatment of pyrethroid permethrin and cypermethrin at higher dosages. Contrary to the present investigation, Garg *et al.*, (2004) [59] reported enhanced ATPase activities after fenvalerate and monocrotophos administration to broiler chicks.

Mitochondria are the powerhouses of a cell, providing much of the cellular ATP. Also, mitochondrial enzymes like succinate dehydrogenase (SDH) participate in many cellular biosynthetic processes. It is also a vital enzyme of the citric acid cycle which catalyzes the reversible oxidation of succinate to fumarate. In the present investigation, it was visualized that this enzyme's reduced activity after treatment of β–cyfluthrin. This can be correlated with reduced activity of

ATPase as both these enzymes are involved in energy metabolism. Pesticides can induce mitochondrial injury, which can disrupt TCA and oxidative phosphorylation, thus hindering the energy production by the system. Results of the current study are consistent with observations of Devi and Gupta (2014) [60], who have noticed reduced succinate dehydrogenase activity in liver and muscle tissues of freshwater fish *Anabas testudineus* after administration of permethrin and deltamethrin for 21 days. Also, Singh *et al.* (2009) [56] reported decreased Succinate dehydrogenase activities in brain tissue of Albino rat (*Rattus norvegicus*) after β-cyfluthrin treatment for acute and sub-acute toxicity studies. Sekhar *et al.* (2010) [61] have also recorded that cypermethrin and sodium fluoride alone as well as synergistically decrease SDH activity in liver and brain tissues after 15 days of treatment in albino mice.

The structural and functional integrity of the plasma membrane can be assessed by the status of biomarkers enzymes activities of ALP (alkaline phosphatase) and ACP (acid phosphatase). Cellular membrane damage might lead to ionic imbalance and thus responsible for the stimulation of lysosomes and liberation of these hydrolytic enzymes. A remarkable decrease was observed in the activity of ALPase in tissue homogenate after β–cyfluthrin administration. In support of our results, Bhushan *et al.* (2013) [53] have also observed decreased ALPase activity in liver tissue of Wistar rats treated with cypermethrin and β-cyfluthrin in dose and duration-dependent manner. ACPase activity in the current study was observed to increase in β-cyfluthrin administered animals after 21 days of treatment. Similar elevated ACPase activity was also reported by Desai *et al.* (2017) [62] in the liver after administration of deltamethrin for 14 and 21 days in Swiss albino mice. Agrawal *et al.* (2019) [63] have also observed a significant upsurge in ACPase activity in female Wistar albino rats after one week of beryllium treatment in hepatic and renal tissues, which suggested enhanced tissue catabolism and cellular autophagy leading to tissue damage [64].

Moreover, ameliorative data of the present study has also demonstrated the protective efficacy of *Punica granatum* juice against β-cyfluthrin induced toxicity in reproductive tissues of male Swiss albino mice. Administration of PJ with pyrethroid toxicant (β-cyfluthrin) effectively increased the body weight and testis weight. As PJ is rich in ellagic acid and other antioxidants, which can reduce the oxidative stress induced by the pesticide and improves cellular function, growth and thus can increase body weight and tissue weight (Testis). Also, supplementation of PJ along with β-cyfluthrin showed the mitigative effect of PJ by enhancing the Protein content, ATPase, SDH, and ALPase activity in Testis. Moreover, PJ has also been found to reduce the ACPase activity significantly after its co-administration with β-cyfluthrin. Therefore, the amelioration with *Punica granatum* juice has been effective in curbing the β-cyfluthrin induced toxicity.

CONCLUSION: The results discussed above reveal that persistent use of β-cyfluthrin (21 days) causes alterations in terms of gravimetric and metabolic (biochemical) indices in mammalian reproductive tissue. Most of the toxic manifestations can be prevented by using a mitigating agent like *Punica granatum* juice against β-cyfluthrin induced testicular toxicity. The ameliorative property of *Punica granatum* could be attributed to its rich anti-oxidant property. Therefore, it is suggested to farmers, and occupational workers exposed continuously to this pesticides, to include *Punica granatum* in their routine diet, which can protect them from deleterious effect of the pesticide on reproductive toxicity.

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