Advances in Bioresearch
Adv. Biores., Vol 14 (1) January 2023: 108-118

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.1.108118

Advances in Bioresearch

ORIGINAL ARTICLE

Amleiorative Role of Pumpkin Seed Extract on Reproductive Toxicity Induced by Quizalofop-P-Ethyl on Swiss Albino Male Mice

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ABSTRACT

Reproductive effects from herbicide exposure in mammals and the ecological assessment of chronic exposure is currently seen to be matter of concern. Quizalofop-p-ethyl is a herbicide used for controlling grass weeds in various crops such as soybeans, peanuts, cotton, potatoes, sugar beets and flax. The reproductive toxicity of herbicide is well studied and an attempt has been made to investigate the ameliorative role of pumpkin seed extract (PSE) against Quizalofop-p-ethyl toxicity in swiss albino male mice. Male mice were given oral doses of 1/25th of LD50 of the Quizalofop-p-ethyl (587.5 mg/kg), 1/50th LD50 (1175 mg/kg) and simultaneously the mice were administered with PSE at dose of 300 mg/kg dissolved in water for 30 and 60 days respectively. Symptomatic behavioural changes were noticed, body weight and food consumption was decreased gradually. Treated mice group at 60 days showed significant decrease in testis, epididymis and seminal vesicle weight as compared to group which was given 30 days treatment. The biochemical analysis shows significant decrease in protein, fructose and sialic acid values at both doses levels 587.5mg/kg and 1175mg/kg for 30 and 60 days, increase in cholesterol and glycogen level has been seen at both days of treatment. In contrast, PSE simultaneous administration with Quizalofop-p-ethyl effectively elevated the weight in the organs of mice. The biochemical analysis show increase in protein, fructose and sialic acid values and decrease in cholesterol and glycogen level at 60 days of treatment. The result suggested that by incorporation of PSE, can reduce the reproductive toxicity in swiss albino male mice.

Keywords:-*Quizalofop-p-ethyl, Pumpkin Seed Extract, Biochemical analysis, Reproductive toxicity,Swiss albino male mice*

Received 04.10.2022 Revised 20.11.2022 Accepted 19.12.2022

How to cite this article:
Lata S and Samiksha S. Amleiorative Role of Pumpkin Seed Extract on Reproductive Toxicity Induced by Quizalofop-P-Ethyl on Swiss Albino Male Mice. Adv. Biores. Vol 14 [1] January 2023.108-118

INTRODUCTION

In both developing and industrialized countries, environmental and occupational herbicide pollution is a common problem and may contribute to multi-organ toxicity in man and animals. Quizalofop-p-ethyl is a selective phenoxy herbicide and is used to eliminate or control annual or perennial grass weeds in potatoes, sugar beets, vegetables, cotton and flax [1].Trade name for Quizalofop-p-ethyl is Targa Super, Assure 2, Matador, Leopard etc. So, these toxic agents have ability to cause potential harm to an organism such as bioaccumulation, issues of carcinogenicity, teratogenicity, mutagenic effects and the impact on reproduction. Infertility is one of the major health problems in life and approximately about 30 % of this problem is due to male factors [2]. Several factors (toxicant in environment, smoking, stress and drinking alcohol and increasing age) and diseases (e.g. coronary heart diseases, diabetes mellitus and chronic liver diseases) can interfere with the process of spermatogenesis, reduce semen quantity and quality and decrease male fertility. Insufficient intake of vitamins has been reported to produce deleterious effects on the process of spermatogenesis and production of normal sperms [2-6]. Moreover, dietary intake of antioxidants from natural products can protect sperm DNA from oxidative stress in the mice testis [7]. Pumpkin (Cucurbita pepo) is a leafy green vegetable; it belongs to the Cucurbitaceae family. Pumpkin seed extract (PSE) has many antioxidants and beneficial nutritional supplements. PSE contains high

amounts of vitamin E and tocoferol. It also contains considerable amounts of palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1) and linoleic (C 18:2). Pumpkin seed extract (PSE) is a natural product used in folk medicine for the treatment of hypertension and atherosclerosis [8]. It is rich in many antioxidants, essential fatty acids, amino acids (especially tyrosine and L phenylalanine), phytosterols (e.g. β-sitosterol), β-carotenes, lutein and selenium [9, 10]. Pumpkin seeds contain L-tryptophan, omega-6 and -3 fatty acids [11] and very high concentration of vitamin E [12]. The antioxidant property of PSE could enhance male reproductive health [13]. Pumpkin seeds extract had been used to improve sexual performance, sexual sensation, and copulatory efficiency (Gundidza et al., 2009). In fact vitamins are essential to maintain normal metabolic processes, and homeostasis in the body. Vitamin E is low molecular mass antioxidants that scavenge or quench free radicals (Janisch et al., 2005). Vitamin E (α-tocopherol) is a fat soluble vitamin which regulates oxidation processes in the body as it acts as a powerful antioxidant. [14-16]. So, the present study was therefore designed to investigate the protective effect of pumpkin seed extract against Quizalofop-p-ethyl (a herbicide) induced testicular toxicity in swiss albino male mice.

MATERIAL AND METHODS

HERBICIDE: Quizalofop-p-ethyl was purchased from local market of Jaipur by trade name Targa super.
PUMPKIN SEED: Pumpkin seeds were purchased from True Elements, Amazon India.

Preparation of Pumpkin seeds extract

For the preparation of 80% ethanolic extract of Pumpkin seeds, they were washed, dried and grained into the fine powder mechanically and then subjected to Soxhlet apparatus for extraction with 80% ethanol. Under Soxhlet extraction procedure, 100gm of powdered seeds was taken in 500ml of 80% ethanol. It was heated for 5-6 hour at 60ºC. The extract obtained was filtered using Whatman no.1 filter paper and then evaporated to dryness under reduced pressure. The dried extract was stored at 4ºC.The dried ethanolic extract was characterized by GC-MS from Central analytical Facility, Manipal University, Jaipur. The ethanolic extract of seeds was orally administered to mice at a dose of 300mg/kg b.w. and this dose was selected according to the Aghaei*et al*, 2015.

ANIMAL: Healthy Swiss Albino male mice (25-30 gm) were procured from the animal house of IIS (deemed to be University), Jaipur. The usage of mice was approved by Institutional animal ethical committee (Approval No. IAEC/2019/II/3). A standard pellet diet was given to mice and water was provided *ad libitum*. A 12 hour light/ 12 hour dark schedule, controlled temperature and humidity conditions were maintained in animal house.

EXPERIMENTAL DESIGN AND GROUPING:-

The male mice were divided into 10 groups and in each group 6 animals were taken Group 'A' served as control for 30 days treatment, Group 'B' 1/25th LD50 of Quizalofop-p-ethyl i.e. dose of 587 mg/kg was given, Group 'C' 1175 mg/kg i.e. 1/50th LD50 of Quizalofop-p-ethyl, Group 'D' 1/25th LD50 of Quizalofop-p-ethyl i.e. dose of 587 mg/kg was given with simultaneous administration of PSE at dose of 300mg/kg and Group 'E' 1/50th LD50 of Quizalofop-p-ethyl i.e. dose of 1175 mg/kg was given with simultaneous administration of PSE at dose of 300mg/kg - was given for 30 days. For 60 days Group 'F' served as control, Group 'G' 1/25th LD50 of Quizalofop-p-ethyl i.e. 587 mg/kg, Group 'H' is of 1175 mg/kg, Group 'I' 1/25th LD50 of Quizalofop-p-ethyl i.e. dose of 587 mg/kg was given with simultaneous administration of PSE at dose of 300mg/kg and Group 'J' 1/50th LD50 of Quizalofop-p-ethyl i.e. dose of 1175 mg/kg was given with simultaneous administration of PSE at dose of 300mg/kg were given treatment for 60 days. The treatment was given orally through intubation tube and mice were dissected on 31st and 61st day respectively.

METHODS OF BIOCHEMICAL ANALYSIS:-

Biochemical tests were performed by respective methods i.e.Protein estimation [17], Cholesterol [18], Fructose, Sialic acid [19], Glycogen [20]. Markers of Steroidogenesis: 3β Hydroxysteroid dehydrogenase and 17β Hydroxysteroid dehydrogenase [21]and sperm parameters were: Sperm Motility and Sperm count, Sperm viability and sperm deformities [22].

STATISTICAL ANALYSIS:-

Data was analyzed and compared by using SPSS software. And the difference was calculated by their P values, significant if the (*P<0.05) and highly significant (**P<0.01) when experimental groups are compared to relative control and significant if the (@P<0.05) and highly significant (@@P<0.01) when compared between the groups.

RESULTS

The male mice were administered with two doses of Quizalofop-p-Ethyl. Experimental animal showed lethargy, red nose, sneezing, rubbing of nose and decrease in food intake.

Table 1:- Effects of administration of Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) on relative body weight, testis weight, epididymis weight and seminal vesicle weight of *Swiss Albino* male mice after 30 and 60 days of treatment.

PARAMETER	TREATMENT	30 DAYS	60 DAYS
BODY WEIGHT (gm)	CONTROL	32.25±1.02	35.41±2.03
	QUIZALOFOP-P-ETHYL TREATMENT (587.5 MG/KG)	28.36±2.16	22.48±0.81 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	27.38±1.31 **	20.12±0.98 **
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	31.81±2.34	32.55±1.04##
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	25.48±1.14	29.30±1.05^^
TESTIS WEIGHT (mg)	CONTROL	124.33±0.58	124.29 ± 0.49
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	120.47 ± 1.38	111.95 ± 1.23 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	115.05 ± 1.15@	98.18 ± 0.56 **
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	128.4±0.83	124.09±0.71##
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	121.59±0.68^	120.7±0.30^^
EPIDIDYMIS WEIGHT (mg)	CONTROL	12.39 ± 0.05	15.5 ± 0.42
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	12.14 ± 0.26	11.95 ± 0.15 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	14.05 ± 0.50@	9.18 ± 0.11 **
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	14.4±0.22	17.09±2.08##
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	15.59±0.53	13.76±0.24^^
SEMINAL VESICLE WEIGHT (mg)	CONTROL	149.11 ± 0.70	147.62 ± 1.04
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	147.93 ± 0.43	138.35 ± 2.48 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	143.1 ± 0.76 *	135.4 ± 0.10 **
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	150.18±0.24	145.74±0.50##
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	148.14±0.31	143.15±0.37^^

Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) resulted in highly significant decrease (**P<0.01) in body weight of mice after 30 and 60 days of treatment as compared to respective controls. Simultaneous administration of PSE (300 mg/kg b.w.) with Quizalofop-p-ethyl at dose of (587.5 mg/kg) for 30 and 60 days was given to mice, resulted in significant increase (#P<0.05) and (##P<0.01) in body weight of mice as compared to their individual exposure whereas when PSE (300 mg/kg b.w.) administered with Quizalofop-p-ethyl at dose of (1175 mg/kg) for 30 and 60 days, significant increase (^^P<0.01), (@@P<0.01) was observed in the body weight of testis.

Significant decrease (**P<0.01) in the weight of testis was seen after the mice was treated with Quizalofop-p-ethyl at dose of (587.5 mg/kg) for 60 days as compared to the control mice. Similarly mice treated with Quizalofop-p-ethyl at dose of (1175 mg/kg) showed significant decrease (**P<0.01) in weight of mice testis at 30 and 60 days as compared to their respective controls.

When the simultaneous administration of PSE (300 mg/kg b.w.) was given with Quizalofop-p-ethyl at dose of (587.5 mg/kg) resulted in a significant increase (##P<0.01) in the weight of testis at 60 days. PSE (300 mg/kg b.w.) when given along with the Quizalofop-p-ethyl at dose of (1175 mg/kg) to the mice leads to significant elevation in the weight of testis (^^P<0.01) and (@@P<0.01) at 60 days of treatment.

Highly significant decrease (**P<0.01) in epididymis weight was observed as compared to their relative control at 60 days of treatment. Significant decline (@P<0.05) in epididymis weight has seen in Group III as compared to Group II. Simultaneous administration of PSE (300 mg/kg b.w.) with Quizalofop-p-ethyl at dose of (587.5 mg/kg) (##P<0.01), Quizalofop-p-ethyl (1175 mg/kg) (^^P<0.01) combination for 60 days resulted in a significant increase in weight of epididymis as compared to the individual exposure.

After 30 days of treatment significant decrease (*P<0.05) in the seminal vesicle weight as compared to control groups, similarly at 60 days of treatment highly significant decrease (**P<0.01) in the seminal vesicle weight was seen when compared to relative control. When the simultaneous administration of PSE (300 mg/kg b.w.) was given with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg) resulted in a significant increase (##P<0.01), (^^P<0.01) and (@@P<0.01) in the weight of seminal vesicle after 60 days of treatment.

Table 2:- Effect of administration of dose of Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) on various biochemical parameters in testis of *Swiss Albino* male mice (n=6).

PARAMETER	TREATMENT	30 DAYS	60 DAYS
TOTAL PROTEIN (mg/g)	CONTROL	24.26 ± 1.41	23.94 ± 0.96
	QUIZALOFOP-P-ETHYL(587.5 MG/KG)	10.5 ± 0.97	11.85 ± 1.22 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	8.69 ± 2.49 *	5.76 ± 0.82@
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	19.06±2.3	19.02±2.8##
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	18.29±3.32^	16.59±2.95^^
SIALIC ACID LEVEL (µg/mg)	CONTROL	22.92 ± 1.19	20.77 ± 0.70
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	10.64 ± 0.67 **	6.83 ± 0.70 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	9.77 ± 1.59 **	4.80 ± 0.69 **
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	16.56±0.55	17.01±0.39##
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	12.35±1.51	19.88±1.80^^
TOTAL CHOLESTEROL (mg/100mg tissue weight)	CONTROL	62.12 ± 5.01	56.12 ± 6.89
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	83.48 ± 4.43	123.68 ± 18.2 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	89.56 ± 2.38 *	115.32 ± 7.22@
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	70±2.54	61.64±2.77##
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	73.90±2.11	65.92±5.92^^
GLYCOGEN (µg/100mg)	CONTROL	355.21 ± 2.50	372.84 ± 2.16
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	372.17 ± 10.35 *	416.22 ± 7.22 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	396.72 ± 12.73 *	445.78 ± 3.58@@
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	354.28±4.78	368.85±6.73##
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	388.26±8.73	381.09±3.13^^
FRUCTOSE LEVEL in seminal vesicle (µg/mg)	CONTROL	34.95 ± 2.11	29.35 ± 0.52
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	25.3 ± 0.94 *	19.76 ± 0.78 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	16.89 ± 1.78 **	10.97 ± 0.70@
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	28.24±0.65	29.20±1.57##
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	24.25±1.88	18.69±0.73^

The values of protein level showed significant decline (*P<0.05) in Quizalofop-p-ethyl group at dose (1175 mg/kg) after 30 days of treatment. Highly significant decrease (**P<0.01) was observed in protein activity at 60 days of treatment with Quizalofop-p-ethyl at both the doses. No significant change was observed between the groups at 30 days of treatment. But significant decline (@P<0.05) in protein level has seen in Group III at 60 days of treatment. But when 300 mg/kg dose of PSE was given simultaneously with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg), significant increase in protein levels was observed at 30 days (^P<0.05) and at 60 days (##P<0.01) and (^^P<0.01) was seen.

Quizalofop-p-ethyl treated mice resulted in highly significant decrease (**P<0.01) in sialic acid level in the testis after 30 and 60 days of treatment as compared to respective controls. No significant change was

observed between the group III and group II at 30 and 60 days of treatment. Simultaneous administration of PSE (300 mg/kg b.w.) with Quizalofop-p-ethyl at dose of (587.5 mg/kg) at 30 and 60 days when given to mice, resulted in increase in sialic acid levels as compared to their individual exposure whereas when Pumpkin seed extract (300 mg/kg b.w) administered with Quizalofop-p-ethyl at dose of (1175 mg/kg) for 30 and 60 days, significant increase (^^P<0.01), (##P<0.01) was observed in the level of sialic acid.

When Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) was administered orally for 30 days, significant increase (*P<0.05) in glycogen level was observed as compared to control values. But when the treatment was continued for 60 days, highly significant increase (**P<0.01) in glycogen level was observed. Highly significant increase (@@P<0.05) in glycogen level has observed in Group III when compared with Group II at 60 days of treatment. Simultaneous administration of PSE (300 mg/kg b.w.) with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg) resulted in a significant decrease in glycogen level after 30 and at 60 days of treatment (##P<0.01) and (^^P<0.01) as compared to their individual treated groups.

The changes in fructose level of seminal vesicle of male mice is depicted in table 2. Significant decrease was observed in fructose level after 30 days of Quizalofop-p-ethyl (587.5 mg/kg) (*P<0.05) and at dose 2 when the treatment was further continued for 60 days, the fructose level showed highly significant decrease (**P<0.01) in Group III when compared to respective control. Significant decrease (@P<0.05) in fructose level has been observed in Group III when compared to Group II. When PSE extract (300 mg/kg b.w.) was simultaneously given with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg) to the mice for 60 days, significant increase (##P<0.01) and (^P<0.05) sin the fructose level was observed

Table 3:- Effect of administration of dose of Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) on markers of steroidogenesis after 30 and 60 days of treatment in *Swiss Albino* male mice (n=6).

PARAMETER	TREATMENT	30 DAYS	60 DAYS
3β -HSD (nano mole of and-rostenedione formed/mg protein/min)	CONTROL	1.86±0.004	1.94±0.006
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	1.75±0.007	1.35±0.02 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	1.65±0.03 *	1.16±0.02 **
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	1.84±0.005	1.77±0.08#
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	1.78±0.003	1.59±0.04
17β -HSD (nano mole of and-rostenedione formed/mg protein/min)	CONTROL	1.87±0.004	2.01±0.05
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	1.75±0.003	1.48±0.02 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	1.59±0.07 *	1.33±0.001 **
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	1.83±0.001	1.95±0.08#
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	1.36±0.007	1.50±0.003#

3β -HSD and 17β -HSD levels has significantly decreased when compared to respective control after 60 days of treatment. Highly significant decrease has been seen at dose 1 and dose 2 at 60 days when compared to respective control but significant decrease has been seen in group III when compared to group II. Simultaneous administration of PSE (300 mg/kg b.w.) with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg) resulted in a significant increase in glycogen level at 30 and at 60 days of treatment (#P<0.05) as compared to their individual treated groups.

Table 4:- Effect of administration of the Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) on sperm parameters of *Swiss Albino* male mice (n=6).

PARAMETER	TREATMENT	30 DAYS	60 DAYS
SPERM MOTILITY (%)	CONTROL	82.66±0.90	81±3.64
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	45.87±1.43 **	29.06±3.50 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	35.86±0.80 **	27.86±3.31 **
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	77.06±1.69##	60.4±4.17##
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	65.6±3.58^^	67.8±4.50^^
SPERM VIABILITY (%)	CONTROL	69.06±3.96	71.46±4.44
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	30±3.79 **	23.4±3.19 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	25.33±4.25 **	18.4±1.35@
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	41.2±2.44##	45.86±3.56#
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	37.4±1.59^	38.13±1.90^
SPERM COUNT (million/ml)	CONTROL	43.45±1.31	42.16±2.75
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	28.85±0.98 **	20.9±0.23 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	21.48±0.79@	18.33±2.03 **
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	39.53±1.16#	35.05±1.70##
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	30.68±2.32^	33.65±2.11^^
SPERM ABNORMALITY (%)	CONTROL	70±1.31	76.83±0.36
	QUIZALOFOP-P-ETHYL (587.5 MG/KG)	54.16±1.54 *	38.5±1.03 **
	QUIZALOFOP-P-ETHYL (1175 MG/KG)	36±0.38@	22.16±1.62 **
	QUIZALOFOP-P-ETHYL (587.5 MG/KG) + PSE (300 MG/KG)	61.5±2.96	55.16±0.95##
	QUIZALOFOP-P-ETHYL (1175 MG/KG) + PSE (300 MG/KG)	48±0.52	39.66±0.35^

Highly significant reduction (**P<0.01) in the cauda epididymal sperm motility was seen after the mice was treated with the both doses of Quizalofop-p-ethyl after 30 and 60 days of treatment as compared to control values. No significant change was observed between the group II and group III. When the simultaneous administration of PSE (300 mg/kg b.w.) was given with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg) resulted in a significant increase (##P<0.01) and (^^P<0.01) in the number of cauda epididymis motile sperms at 30 and 60 days of treatment.

When mice was treated with Quizalofop-p-ethyl at dose 587.5 mg/kg and 1175 mg/kg for 30 and 60 days. Highly significant decrease (**P<0.01) in the cauda epididymal sperm viability was seen as compared to the control values. Significant decrease (@P<0.05) is seen in Group III at 60 days when compared to Group II. When dose of 300 mg/kg b.w. PSE was given simultaneously with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg) for 30 and 60 days, significant increase (#P<0.05), (^P<0.05) and (@P<0.05) in the number of viable sperms was observed as compared to the individually treated groups

In the cauda epididymal sperm count highly significant decline (**P<0.01) was observed after 30 and 60 days at dose 1 and dose 2 of Quizalofop-p-ethyl treated mice as compared to control value. Significant decrease (@P<0.05) has been observed in Group III at 30 days when compared to Group II. When the treatment of 300 mg/kg b.w. PSE with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg) was given to mice for 30 days showed significant increase (#P<0.05) and (^P<0.05) in the number of sperms as compared to their individual exposure. When the treatment was further continued for 60 days treatment of Pumpkinseed extract with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg) showed significant recovery (##P<0.01) and (^^P<0.01) in the sperm count as compared to their individual treated groups for 30 and 60 days.

Quizalofop-p-ethyl at dose 587.5 mg/kg and 1175 mg/kg led to sperm abnormality in mice. Statiscally highly significant decrease (**P<0.01) was observed in the number of normal sperms when mice treated with Quizalofop-p-ethyl for 30 and 60 days. Significant decrease (@P<0.05) was observed in Group III

when compared to Group II at 30 days. Few abnormalities of sperms like two headed sperms, banana shaped head sperms, long tailed sperms and double headed sperms were observed in the Quizalofop-p-Ethyl treated mice. No significant recovery was observed in number of normal sperms after treated with PSE (300 mg/kg b.w.) along with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg) at 30 days of treatment as compared to their individually treated groups. Simultaneous administration of 300 mg/kg b.w. PSE along with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg) was given to mice for 60 days resulted in significant increase (##P<0.01) and(^P<0.05) in number of normal sperms as compared to their individual treated groups.

DISCUSSION

Although pesticides are double edge sword it has its own benefits as well as some drawbacks and having potential toxicity to all life forms. The continuous use may lead to several deadly, hazardous diseases (Deshmukh *et al;* 2015). In this present study Quizalofop-p-ethyl treated mice show decrease in body weight and organ weight, eye discharge and decrease in food intake. Similar results such as decrease in food consumption, body weight, testis weight were observed in rats at dose of 433mg/kg and 1300mg/kg of Quizalofop-p-ethyl [23].

The study indicates that body weight and organ weight (testis, epididymis and seminal vesicle) of Swiss Albino male mice has significantly decrease in experimental group treated with both doses of Quizalofop-p-ethyl (587.5 mg/kg) and (1175 mg/kg) at time interval of 30 and 60 days of treatment as compared to relative control group and within the groups, similar results were seen by Jian-Xi *et al.,* 2005[24]in which he reported that their was decrease in organ coefficient of testis, seminal vesicle and epididymis when rats were treated with Quizalofop-p-Ethyl for 3 months of time interval at dose of 433mg/kg and 1175mg/kg. The current study was designed to investigate the protective effect of pumpkin seed extract (PSE) against Quizalofop-p-ethyl which induced reproductive toxicity effecting biochemical parameter and sperm parameters. Results of this study revealed that oral administration of Quizalofop-p-ethyl to male mice (587 mg/kg and 1175 mg/kg) for consecutive 30 and 60 days induced reproductive toxicity. The toxic effect of Quizalofop-p-ethyl was manifested by decreased weight of testes, lowered semen quantity and quality. These results correlate with those reported by Hamza and Amin [25] Wilke and Utley [26], Walker et al. [27], Soliman and Abdel Meguid[28] and Bairy et al. [29]. They found that oral administration of Quizalofop-p-ethyl to mice decreased relative weights of testes and epididymis and reduced sperm numbers and viability. Concerning pumpkin seed extract (PSE), the previous studies reported that PSE is rich in many antioxidants and beneficial nutritional components such as essential fatty acids, amino acids, phytosterols, -carotenes, lutein and selenium [30,31]. PSE also contain very high concentration of vitamin E which acts as a powerful antioxidant. Essential fatty acids are required constituents of health of cell membrane as they maintain the fluidity of cell membranes [32]. This result agreed with Alan, 2006 said that pumpkin seed extract is excellent source of magnesium, phosphorus, manganese, copper, iron and zinc which vital in growth thus increasing body weight, testis weight, epididymis weight and seminal vesicle weight.

[33] The protein level of testis in our study was decreased as compared to control group at 60 days of treatment suggesting defective spermatogenesis, similar findings were seen by Deshmukh *et al;* 2015. Similarly decline in protein level and increase in cholesterol level has been seen in rats which received the dose of 62.5 or 125mg/kg of Pirimiphos-methyl for 90 days [34]. Cholesterol is mandatory for the regular testicular activity. It is a necessary basic component of all cell membranes and is an initiator of steroid hormones and for biosynthesis of bile acid (Chen *et al.,* 2011). Significant increase in cholesterol level of testis has been observed when treated with Quizalofop-p-ethyl at dose of (587.5 mg/kg) and (1175 mg/kg) for 30 and 60 days indicating metabolism has been affected. In 2010 it has been reported that the cells in the testis need cholesterol for biogenesis of membrane, cell signalling and it act as a precursor for synthesis of androgen [35]. In the present study, cholesterol levels in testis was increased after treatment with Quizalofop-p-ethyl in mice for 30 and 60 days. Increased cholesterol level in testes of male mice when treated with cypermethrin [36].

Sialic acid is a sialo mucopolysaccharide which is required for maintaining the structural integrity of membranes of spermatozoa and also aid in maturation of their membrane [37]. The amount of sialic acid in testis and epididymis is associated with androgen level [38]. Sialic acid also forms a part of glycoprotein and glycolipids [39]. Significant decline in levels of sialic acid in testis is observed in the present study when compared to their respective controls which suggested that structural integrity of acrosomal membrane of spermatozoa is affected. Sialic acid and protein content of testis were decreased and cholesterol level was increased in dose dependent manner in mancozeb treated animals [40]. Similarly biochemical profile of the testis revealed a significant decline in the contents of sialic acid,

whereas significant increase in cholesterol level was observed when treated with malathion [41]. Fructose is one of the most important markers of seminal vesicular function. Fructose helps in the spermatozoa metabolism and provides energy to the sperms and increase in sperm motility [42]. The formation and secretion of fructose by seminal vesicle depends upon the exudation of testosterone from the Leydig cells of the testis. The values of fructose level in seminal vesicle has significantly decreased after 30 and 60 days of treatment which depicts towards impairment in the secretory function ascribed to androgen depletion. Glycogen is the storage form of glucose in animals and is considered as the energy source in the testis and epididymis. For maturation and functioning of gonads testicular glycogen plays a crucial role [43]. It is also a source of glucose 6- phosphate required for pentose phosphate pathway **[44]**. Significant increase in glycogen level was observed in control values and between the groups. Biochemical parameters are good diagnostic tool for reproductive toxicity. On contrast when Pumpkin seed extract (300mg/kg) was simultaneously administered with Quizalofop-p-ethyl it elevates the level of protein, sialic acid and fructose whereas decreases cholesterol and glycogen level because of presence of sterols and polyunsaturated fatty acids in pumpkin seed extract making it an excellent for pharmaceutics. Pumpkin flavonoid has anti-inflammatory activity and anti-microbial activity. Pumpkin polysaccharide exhibits higher cytoprotective effect, indicating that could enhance the cytoprotective effect [45]. Flavonoids, one of the phytoconstituent were found in pumpkin which contribute antibacterial effects as recorded in different studies [46].

Increase in cholesterol level is interrelated with decline in the activities of steroidic enzymes (3β hydroxysteroid dehydrogenase and 17β hydroxysteroid dehydrogenase) which disturb the testicular steroidogenesis. Testicular androgenesis is controlled by two rate limiting enzymes 3β -HSD and 17β -HSD (Ghosh *et al.,* 1989; Ishii-Ohba, 1986). The enzyme 3β-hydroxysteroid dehydrogenase (3β-HSDs) and 17β-hydroxysteroid dehydrogenase (17β-HSDs) are the key enzymes in the biosynthesis of all the steroid hormones. Decrease in level of 3β -HSD and 17β -HSD level has been seen when treated with Quizalofop-p-ethyl at 30 and 60 days of treatment. Similar results were seen in Kostic*et al.,* 2011 suggested that androgen production can inhibit its production in testis and its repression is regulated by 3β-HSD level [47]. The changes in the synthesis of steroid hormones may be due to the changes in activity of steroidogenic enzymes. 3β-HSD is one of the important enzymes in the biosynthesis of androgens and all other physically active steroid hormones [48]. But when Pumpkin seed extract (300mg/kg) was simultaneously administered with Quizalofop-p-ethyl it elevates the level of 3β-hydroxysteroid dehydrogenase (3β-HSDs) and 17β-hydroxysteroid dehydrogenase (17β-HSDs). The presence of selenium in PSE helps and maintain the antioxidant system. Selenium is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes. It has been reported that selenium can improve male fertility due to its antioxidant properties.

Pumpkin seed extract have rich source of zinc, which promote prostate health [49]. Sperm viability significant decrease in live sperm and increase in dead sperm in group treated with Quizalofop-p-ethyl compared to control group , these result agree with Sokol et al., (1985)[50] due to the accumulation of Quizalofop-p-ethyl in many organs and fluids specially the gonads and seminal fluid (Silbergeld, 983)[51]. Group treated with (Quizalofop-p-ethyl and pumpkin seed extract) revealed that significant increase in live sperm and significant decrease in dead sperm compared with group treated only with Quizalofop-p-ethyl. These results agreed with [52]Jamilah, (2013), which attributed to pumpkin seed extract is rich in Zinc plays an important role in the structure of proteins and cell membranes and protect against damage so plays important roles reproduction [53]. The effect of Quizalofop-p-ethyl on sperm abnormality showed significant increase in sperm abnormality in group treated with Quizalofop-p-ethyl compared to control group, these results agreed with Chowdhury,[54], Quizalofop-p-ethyl would induce disruption of spermatogenesis in the testes causing deuteriation of motility and content of sperm as well as abnormalities as reported by Gracia-Leston et al.,[55]. The result of group (Quizalofop-p-ethyl and pumpkin seed extract) agreed with Nkang et al., [56], due to PSE contains B-carotene, a potent free radical quencher, singlet scavenger and lipid antioxidant. These results may be due to pumpkin seed extract is rich in essential fatty acid are required constituents of health of cell membrane and rich in vitamin E which acts as a powerful antioxidant[57].Murkovic et al. [58], who concluded that the antioxidant property of PSE could enhance male fertility in rats. In conclusion, our study has demonstrated that pumpkin seed extract ameliorate Quizalofop-p-ethyl induced testicular damage.

CONCLUSION

It can be concluded that Quizalofop-p-ethyl has the potential to produce reproductive toxic effects and biochemical alterations in swiss albino male mice. Accordingly, strict limitations on the use of this compound must be put. PSE appears to have abundant beneficial properties for applications in food. The

content of compounds such as polyunsaturated fatty acids, essential amino acids, vitamin E, selenium and polyphenols makes PSE a supply to satisfy essential needs in human diet and health maintenance. Data illustrated that PSE can be used as therapeutic agent to attenuate the deleterious effects of Quizalofop-p-ethyl. Subsequently, it can be categorized as edible food ingredient with a high potential of antioxidant activity.

ACKNOWLEDGEMENT
The experimental work was performed under the assistance of Dr. Lata Shahani and I would like to give my sincere thanks to Department of Zoology for providing me the facilities.

CONFLICT OF INTEREST
No conflict of interest

ETHICAL APPROVAL
The study was approved and the work was performed in accordance with the guidelines of Institutional Animal Ethical Committee (Approval No. IAEC/2019/II/3).

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