4 Results and Discussion

The results of the present investigation entitled **"ANTIOXIDANT POTENTIAL OF *Cucurbita pepo* L. (PUMPKIN) SEED EXTRACT IN THE TREATMENT OF STRESS INDUCED MALE INFERTILITY: AN IN VIVO STUDY"** are presented and discussed under the following headings.

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PHASE I

4.1. Nutritional Analysis of *Cucurbita pepo* L. Seeds

4.1.1. Collection and certification of *Cucurbita pepo* L. seeds

Pumpkin (*Cucurbita pepo* L.), a Cucurbitaceae family member, is an exotic crop that thrives in both open field and protected environments. In India, its cultivation has become increasingly popular due to its high yield potential and the health benefits it offers. It thrives in a variety of climatic and soil conditions. The *Cucurbita pepo* L. seeds were sourced from an organic farm in Tudiyalur, located in Coimbatore district. They were subsequently submitted to a taxonomist at the Botanical Survey of India, Southern Regional Centre, Tamil Nadu Agricultural University, Coimbatore, for taxonomical certification. The collected seeds were verified and duly certified, with a voucher number issued for the same.

4.1.2. Analysis of nutrient content in *Cucurbita pepo* L. seeds

Cucurbita pepo L. seeds are among the most widely cultivated and nutritious vegetable seeds globally. They are renowned for their therapeutic properties, attributed to the wide array of bioactive components they contain. Research has demonstrated that *Cucurbita pepo* L. seeds are rich in polyphenols, antioxidants, fiber, vitamins (including β-carotene, vitamin C, and vitamin E), iron, folate, and numerous other micro and macro elements (Badu *et al*., 2020). A nutrient analysis provides valuable insights into the composition of a given sample (Sinkovic and Kolmanic, 2021), and as such, the nutritional composition of selected *Cucurbita pepo* L. seeds was determined

Initially, the proximate composition of the *Cucurbita pepo* L. seeds was assessed serving as a basis for estimating their nutritional content and quality. Raw *Cucurbita pepo* L. seeds were analyzed for energy, carbohydrate, moisture, total ash, protein, fiber, and fat content. The proximate composition of the *Cucurbita pepo* L. seeds, expressed per 100 grams, is presented in a pie chart (Figure 3).

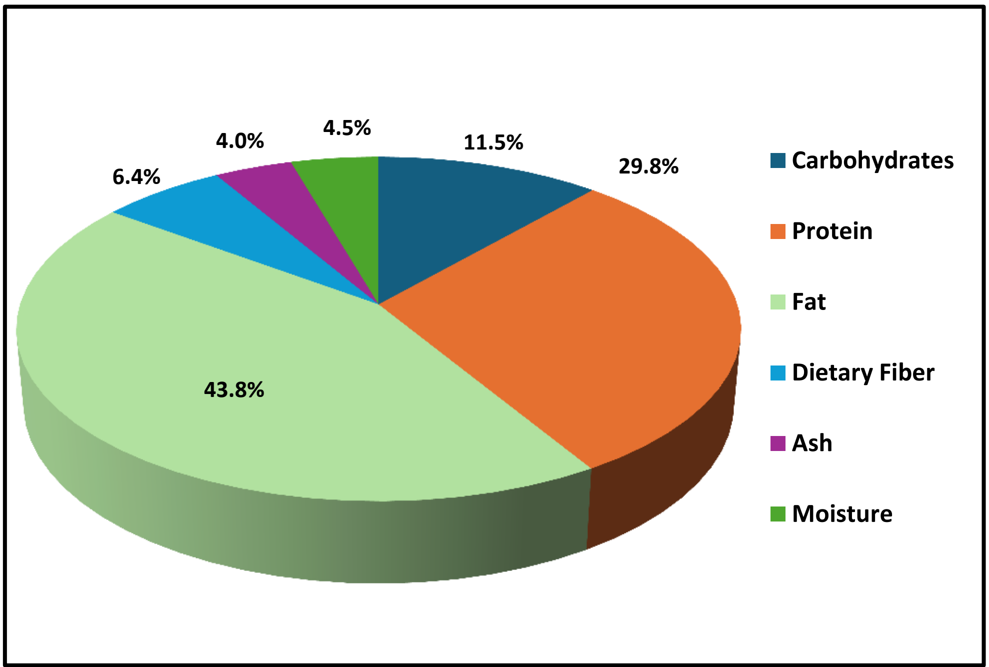

Figure 3: Proximate Composition of *Cucurbita pepo* L. Seeds

Moisture content in food products influences their dry matter content. As moisture levels increase, the yield of dry matter decreases during drying. The present study revealed that *Cucurbita pepo* L. seeds had comparatively low moisture content when compared to other vegetable seeds, such as amaranth seeds (10.6±0.2%) and pomegranate seeds (6.84±0.03%), as reported by Petkova *et al*., (2019) and Abiola, (2018), respectively. The low moisture content indicated the storage benefit of the *Cucurbita pepo* L. seeds, preventing microbial spoilage ensuring a better shelf life. Generally, dried pumpkin seeds with low moisture content are less likely to be attacked by microorganisms (Barros *et al*., 2024). The ash content of *Cucurbita pepo* L. seeds was found to be similar to that of chia seeds (4.45%) (Kibui *et al*., 2018). The ash content reflects the quantity of minerals present in a food material. A sample with a high ash content is likely to contain a greater concentration of various mineral elements, which are anticipated to accelerate metabolic processes and enhance growth and development (Kashyap *et al*., 2022).

Cucurbita pepo L. seeds were found have a high calorific value (559.4 kcal/100 g), significantly contributing to the energy requirements of the human body. The crude fat content indicated how *Cucurbita pepo* L. seeds could be regarded as oilseeds, like sunflower, soybean, and mustard. The fat content of *Cucurbita pepo* L. seeds was close to the value of those grown in the ‘Savanna’ regions of Ghana, as reported by Badu *et al*., (2020). Plant proteins have been studied as a possible substitute for animal proteins due to their cost-effectiveness (Sá *et al*., 2020). Most bodily tissues rely on dietary protein for their natural production and upkeep. The protein content of the sample suggests that *Cucurbita pepo* L. seeds are a valuable source of protein and could help in eradicating protein deficiency. The available carbohydrate content of the seed was found to be low, which doesn’t align with the findings of Ghaffar *et al*., (2018), possibly due to the difference in geographic location where they were cultivated. When compared to the carbohydrate levels of certain traditional sources such as cereals, the sample could not be considered a promising carbohydrate source. The fiber content of the seeds demonstrated that *Cucurbita pepo* L. seeds are excellent sources of fiber. Therefore, *Cucurbita pepo* L. seeds could be recommended for relieving constipation, providing protection against certain cancers, and lowering blood cholesterol levels.

The ash content was further analyzed to quantify the vitamins, as well as certain macro- and micro- mineral content per 100 grams of *Cucurbita pepo* L. seeds, as shown in Table I.

Table I

Vitamins and Minerals Content of *Cucurbita pepo* L. Seeds

Sl. No.	Element	Concentration
1.	Vitamin A (µg)	13.3 ± 0.13
2.	Vitamin C (mg)	2.6 ±0.18
3.	Calcium (mg)	48 ±0.25
4.	Iron (mg)	7.5 ± 0.06
5.	Phosphorous (mg)	900 ± 1.10
6.	Potassium (mg)	450 ± 0.98
7.	Magnesium (mg)	520 ±0.43
8.	Zinc (mg)	8.2 ± 0.12

Note: The data are mean value± standard deviation of triplicate results

The potassium and phosphorus concentrations of the sample indicated that *Cucurbita pepo* L. seeds are excellent sources of these essential minerals. The seeds contained approximately 520 mg of magnesium per 100 grams of the sample, implying that they are an exceptional source of magnesium. The sample also contained zinc and iron in abundance. The calcium content further suggested that *Cucurbita pepo* L. seeds are a rich source of calcium. Additionally, this study revealed that the sample is also a valuable source of vitamin C.

Manshi *et al*., (2023) studied that pumpkins abundantly supply calcium, iron, unsaturated fatty acids, and protein. This is in accordance with the reports of Vidhya *et al*., (2022) and Syed *et al*., (2019), which exposed similar results in the analysis of Cucurbita seeds. Their studies showed that the oil and protein content ranged from 23% to 49% and 21% to 30%, respectively, while this study presented values of 43.8% and 29.8%. Moreover, the nutritional value of Cucurbita seeds varies by species and is also influenced by climatic and geographical factors. These findings definitively confirm that the nutritional content of these seeds is dependent on regional climates.

4.1.3. Antinutrient analysis of *Cucurbita pepo* L. seeds

Antinutritional factors are substances naturally produced in food sources through the normal metabolism of species. They operate through mechanisms such as nutrient inactivation, hindering digestion, or restricting the metabolic utilization of feed, all of which have effects that oppose optimal nutrition. Antinutritional components like phytates, oxalates, and nitrates limit the nutrient absorption of plant-based foods, thereby hindering their bioavailability when consumed (Parimelazhagan, 2016). The antinutrient content of *Cucurbita pepo* L. seeds per 100 grams is shown in Table II.

Table II

Antinutrient Content of *Cucurbita pepo* L. Seeds

Sl. No	Element	Concentration (mg)
1.	Oxalate	0.16±0.05
2.	Phytate	8.24±0.18
3.	Nitrate	2.11±0.04

Note: The data are mean value ± standard deviation of triplicate results

Plants are categorized into low oxalate plants (with oxalate concentrations < 10 mg/d) and high oxalate plants (containing oxalate concentrations ranging from 100 mg/d to over 1000 mg/day fresh weight of the sample) (Abdel-Moemin, 2014). Oxalic acid binds strongly with mineral elements like calcium, potassium, and magnesium, rendering them unavailable for biological functions. A diet high in oxalates can increase the risk of calcium absorption by the kidneys and has been linked to kidney stones (Alelign and Petros, 2018). However, the oxalate concentration in the seed was only 0.16 mg/100 grams, a level too low to pose any health threat. The phytate concentration in the seed was 8.24 mg/100 grams, which is within the safe limit. According to the classification of low/high phytate plants, *Cucurbita pepo* L. can be considered a low phytate plant. The nitrate concentration in the sample was found to be 2.11 mg/100 grams, which is below the World Health Organization's recommended limit of 3.7 mg/kg of body weight.

The results of this study strongly advocate for the daily inclusion of *Cucurbita pepo* L. seeds in the diet for numerous reasons. It is a widely cultivated, affordable, and cost-effective vegetable crop that is nutrient-dense and provide high calorific value. Significantly, these findings suggest that consuming *Cucurbita pepo* L. seeds in adequate amounts can greatly enhance human nutritional status. This makes the seeds a valuable addition to the diet, contributing to family food security and fulfilling nutritional needs. Their consumption can promote normal growth and offer active protection against diseases and malnutrition.

PHASE II

4.2. Phytochemical Analyses of *Cucurbita pepo* L. Seed Extracts

4.2.1. Qualitative phytochemical analysis of *Cucurbita pepo* L. seed extracts

The core of natural product research is the extraction of compounds from plant materials (Chibuye *et al.,* 2024). Preliminary screening of phytochemicals can help identify bioactive compounds, which may ultimately contribute to drug discovery and development (Egbuna *et al*., 2019). The phytochemical compounds of *Cucurbita pepo* L. seed extracts of various solvents such as Chloroform (CF), Ethyl Acetate (EA), Acetone (AC), Methanol (ME) and Aqueous (AQ) were qualitatively analyzed and the results are as displayed in Table III.

Table III

Phytochemical Screening of Various Extracts of *Cucurbita pepo* L. Seeds

Sl. No	Compounds	CF	EA	AC	ME	AQ
1.	Carbohydrates	-	-	-	+	++
2.	Protein	-	+	+	+	++
3.	Phenolics	+	+	+	++	++
3.	Flavonoids	-	+	-	++	+
4.	Alkaloids	-	++	+	++	++
5.	Tannins	+	++	++	++	+
6.	Steroids	-	-	-	-	++
7.	Terpenoids	++	+	++	++	++
8.	Saponins	-	-	+	-	+++
9.	Quinones	+	-	+	-	+

+++: appreciable amounts, ++: moderate amounts, +: trace amounts

The qualitative analysis carried out on *Cucurbita pepo* L. seed extracts revealed that the extracts were enriched with a variety of various medicinally active phytochemicals. From Table III, it could be inferred that among all the extracts, aqueous and methanol extracts of *Cucurbita pepo* L. seeds showed the optimum presence of phytochemicals. Nevertheless, aqueous extract was found to have all the phytochemicals under investigation whereas methanol extract lacked a few. Based on the presence of phytochemicals, the aqueous extract may exhibit more therapeutic properties as compared to other extracts.

The presence of secondary metabolites such as steroids, saponins, carbohydrates, proteins, tannins, alkaloids, flavonoids, terpenoids, and quinones in the aqueous extract of *Cucurbita pepo* L. seeds suggested that the extract may have medicinal importance. This finding is in consistent with the report by Mehmood *et al*., (2012) who identified similar phytochemicals in the aqueous extract of *Cichorium intybus* seeds. Additionally, the result indicated that the aqueous extract contained higher levels of bioactive compounds compared to the other extracts, highlighting its therapeutic potential.

Velu *et al*., (2018) suggested that saponins and glycosides are important classes of secondary metabolites with therapeutic potential. Agati *et al*., (2020) and Kaurinovic and Vastag, (2019) reported that flavonoids are among the most diverse and widely distributed groups of natural compounds, known for their broad spectrum of chemical and biological activities. These include radical scavenging properties, antiallergenic, antiviral, anti-inflammatory, and vasodilatory effects. Polyphenolic compounds, known for their antioxidant activity, play a key role in adsorbing and neutralizing free radicals due to their redox properties (Gomathi *et al*., 2015; Olszowy, 2019). Given the presence of these bioactive compounds, it is likely that the bioactivity of *Cucurbita pepo* L. seed aqueous extract is attributed to their combined effects. Thus, the aqueous extract of *Cucurbita pepo* L. seeds may serve as a promising alternative for treating diseases associated with excessive free radical generation. Consequently, aqueous extract was selected for further studies.

4.2.2. Quantitative phytochemical analysis of *Cucurbita pepo* L. seed aqueous extract

The quantitative phytochemical analysis results for the total phenolics, flavonoid, and alkaloid contents of *Cucurbita pepo* L. seed aqueous extract is presented in Table IV.

Table IV

Quantitative phytochemical Analysis of *Cucurbita pepo* L. Seed Aqueous Extract

Sl. No	Phytoconstituents	Results (mg)
1.	Total Phenolics	32.7 ± 0.89
2.	Total Flavonoids	16.8 ± 0.63
3.	Total Alkaloids	11.3 ± 1.12

All determinations were performed in triplicates

Phenolics are the most prevalent secondary metabolites in the plant kingdom. Due to their ability to serve as radical scavengers, these diverse families of compounds have garnered significant attention as potential natural antioxidants. As noted by Elmastas *et al*., (2015), phenolic compounds function as free radical terminators owing to their antioxidant properties.

The total phenolic content in *Cucurbita pepo* L. seed extract was estimated in terms of Gallic Acid Equivalents (GAE). The result revealed that *Cucurbita pepo* L. seeds had a high concentration of phenolic compounds (32.7±0.89 mg GAE/100 g) in it. Xanthopoulou *et al*., (2009) recorded that pumpkin seed water extract contained the highest concentration of phenolic compounds, accounting for 85–92% of the total extractable phenolics, when compared to other extracts. In addition, Zuhair *et al*., (2013) observed that the choice of extraction solvent significantly impacted antioxidant activity, total phenolic and total flavonoid contents when evaluated the effects of different solvents on the phenolic content and antioxidant properties of two papaya cultivars. Ahin *et al*., (2014) discovered that the water extract of *Prunella grandiflora* exhibited the highest total phenolic content (24.63±0.55 mg GAE/gram of extract) when compared to alcoholic extracts. The elevated phenolic concentration in the aqueous extract was likely due to the presence of proteins and other water-soluble compounds containing phenolic rings. This aligns with previous studies indicating that water/aqueous extracts of *Cucurbita pepo* L. seeds also yield high levels of total phenolics.

Flavonoids are regarded as one of the most imperative classes of natural phenols. They exhibit a broad range of biological and chemical activities, including notable radical scavenging properties. The flavonoid content in aqueous extract of *Cucurbita pepo* L. seeds was evaluated using spectrophotometric method using quercetin as standard. The flavonoid content was expressed in terms of Quercetin Equivalent (mg of QE/100 gram of extract). The total flavonoid content was found to be lower than the phenolic content, which is expected, as flavonoids are a subset of phenolic compounds (Shraim *et al*., 2021).

Alkaloids are naturally occurring chemical compounds having a significant impact on plant medicine because of their vast application. Spectrophotometric method is well acknowledged for its simplicity, sensitivity, and rapid determination. This process is based on the formation of a yellow product when alkaloid reacts with Bromo Cresol Green (BCG) (Warusavithana and Safeena, 2022). The sample was found to have a high alkaloid content of 11.3±1.12 mg AE/100 gram of extract. The current study is probably the first to have reported the total alkaloid content of *Cucurbita pepo* L. seed aqueous extract.

Quantitative phytochemical analysis of aqueous extract of *Cucurbita pepo* L. seeds exhibited remarkable amount of total phenolics, flavonoids, and alkaloids. Linear regression equations obtained from gallic acid, quercetin, and atropine standard curves were used for estimating total phenols, total flavonoids, and total alkaloids, respectively. Plant flavonoids and phenols are widely recognized for their strong ability to scavenge free radicals, offering crucial antioxidant defense to living cells. Polyphenols and flavonoids extracted from plants are utilized in the prevention and treatment of various diseases linked to free radicals. These compounds are regarded as powerful antioxidants due to their capacity to absorb and neutralize free radicals, as well as to quench Reactive Oxygen Species. Therefore, *Cucurbita pepo* L. seeds could be recommended as a natural source of antioxidant agents.

4.2.3. Chromatographic screening of bioactive compounds in *Cucurbita pepo* L. seed extract

Gas Chromatography - Mass Spectrometry (GC-MS)

Identifying biologically active compounds in plants is a crucial aspect of plant research, as it paves the way for further biological and pharmacological investigations (Atanasov *et al*., 2015). GC-MS is an excellent technique for identifying bioactive constituents, such as long-chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino compounds, and nitro compounds, present in plant species. With its high sensitivity and specificity, combined with specific detection methods, GC-MS has become a sophisticated and powerful tool for analyzing various compounds of medicinal significance (Velmurugan and Anand, 2017).

The mass spectrometry data (GC-MS) was interpreted using the NIST database, where the spectrum of the unknown component was compared with that of known components stored in the database. The GC-MS spectrum of *Cucurbita pepo* L. seed extract showed prominent peaks indicating the presence of 17 bioactive compounds (Figure 4). The corresponding compounds, along with their retention time (RT), compound name, molecular formula, match factor (MF), compound nature, and associated biological activities, are summarized in Table V.

Table V

GC-MS Profile of *Cucurbita pepo* L. Seed Extract

RT (min)	Compound Name	Molecular Formula	MF	Compound Nature	Biological Activity
4.1156	Cyclobutylamine	C4H9N	80	amine	Neurotransmission modulator, analgesic, antimicrobial, anti-depressant
4.9488	Glycinamide, N(2)-methyl-	C3H8N2O	89.5	Amide	Antiviral, protein biosynthesis
18.2809	1-Tetradecanol	$C_{14}H_{30}O$	95.7	Fatty acid ester	Anti-inflammatory, antibacterial, antimicrobial
19.8719	Cyclopentane, pentyl-	$C_{10}H_{20}$	87.1	Alkane	Antibiotic, anti-tumour
24.1349	1-Octadecene	$C_{18}H_{36}$	94.3	Alkene	Antioxidant, anticancer, anti-tumour, antibacterial
26.0835	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	96.3	Fatty acid	Antioxidant, lubricant, hypocholesterolemic, anti-androgenic, anti-fungal, haemolytic, nematicide, antipsychotic

RT (min)	Compound Name	Molecular Formula	MF	Compound Nature	Biological Activity
26.4366	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	95.7	Fatty acid ester	Antioxidant, anti-androgenic, pesticide, lubricant, flavour, hypocholesterolemic
27.6275	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	98.2	Fatty acid ester	analgesic, anti-inflammatory
28.0909	10(E),12(Z)-Conjugated linoleic acid	$C_{18}H_{32}O_2$	90.1	Linoleic acid isomer	Anti-carcinogenic, anti-ageing, immunobooster, aids weight loss, anti-atherogenic
31.6256	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	97.8	Fatty acid ester	Antioxidant, antimicrobial, haemolytic
32.3807	Stigmasterol	$C_{29}H_{48}O$	93.1	Steroid	Antioxidant, anticancer, anti-arthritic, diuretic, anti-inflammatory, antimicrobial, anti-asthma, anti-diabetic, thyroid inhibiting properties, precursor of progesterone
33.7673	.gamma.-Sitosterol	$C_{29}H_{50}O$	90.6	Phytosterols	Antidiabetic, antioxidant, antihyperlipidemic,

RT (min)	Compound Name	Molecular Formula	MF	Compound Nature	Biological Activity
					anticancer, immunobooster, supports prostate health
34.2436	Squalene	$C_{30}H_{50}$	96.8	Triterpene	Antioxidant, anti-tumour, anti- inflammatory, cancer and chemo preventive, anti-ageing, immunostimulant, detoxifier, diuretic, skin emollient, gastro and hepatoprotective
34.7030	Undec-10-ynoic acid, tetradecyl ester	$C_{25}H_{46}O_2$	67.7	Fatty acid ester	Antioxidant, anticancer, anti-inflammatory, antimicrobial
36.0680	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	$C_{30}H_{50}O$	63.0	Triterpenoid	Anti-inflammatory, antimicrobial, antiarthritic, anti-tumour, anti-protozoal, chemo-preventive
36.8101	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	96.9	Vitamin E compound	Antioxidant, anti-inflammatory, anti-ageing, anti-cancerous, anti-degenerative, cardio and prostate protective, vasodilator,

RT (min)	Compound Name	Molecular Formula	MF	Compound Nature	Biological Activity
					antispasmodic
37.1965	.gamma.-Sitostenone	$C_{29}H_{48}O$	66.8	Phytosterols	Antioxidant, antimicrobial, anti-inflammatory, hypolipidemic, neuroprotective

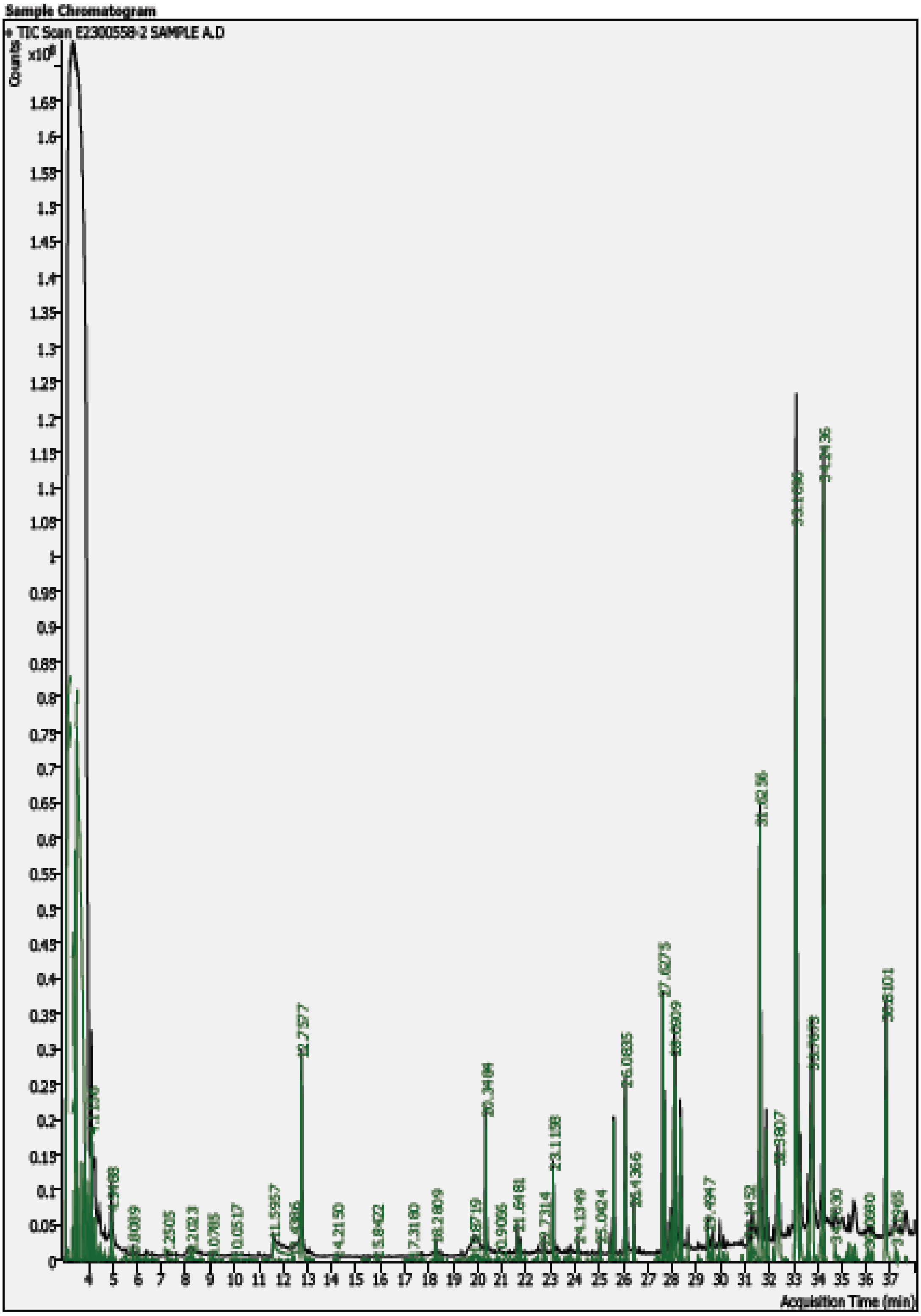

Figure 4: GC-MS Chromatogram of *Cucurbita pepo* L. Seeds

GC-MS analysis discovered a significant presence of compounds such as hexadecanoic acid, octadecane, squalene, and other derivatives. Both hexadecanoic and octadecanoic acids have been documented for their antioxidant and anti-inflammatory properties (Asif *et al*., 2022), as well as their anticancer (Lee *et al*., 2018) and antiapoptotic effects (Abdalla *et al*., 2020). The findings related to the active components of *Cucurbita pepo* L. seed extract are consistent with other studies that have identified significant levels of free fatty acids, including oleic, linoleic, palmitic, and stearic acids, in pumpkin oil (Sener *et al*., 2007; Badr *et al*., 2011).

Pumpkin seed oil is commonly used as a nutritional supplement. It has rich content of omega-3, omega-6, and omega-9 (polyunsaturated) fatty acids, proteins, as well as β-carotenes, lutein, carotenoids, β- and γ-tocopherols, phytosterols, chlorophyll, and trace minerals like zinc and selenium (Majid *et al*., 2020). Additionally, pumpkin seed extracts are utilized as an adjunct for immunomodulation, promoting reproductive health, and supporting the treatment of various diseases (Shaban and Sahu, 2017). Furthermore, the omega-3 and omega-6 essential fatty acids found in pumpkin seed oil play a crucial role in maintaining healthy brain and body functions, and are also beneficial for preventing and managing bladder and prostate issues (Wal *et al*., 2024).

Stigmasterol, an unsaturated plant sterol, serves as a precursor in the production of semi-synthetic progesterone, a vital hormone that regulates physiological processes and aids tissue repair, especially in relation to estrogen effects. It also acts as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. Additionally, stigmasterol is a precursor to vitamin D3 (Bakrim *et al*., 2022). Moreover, it has been found to contain high levels of tocopherol, which contribute to its antioxidant activity, potentially reducing lipid peroxidation (Liang *et al*., 2020).

Squalene is a lipophilic isoprenoid triterpene that plays a key role in the antioxidant properties of pumpkin oil, helping to protect against membrane lipid peroxidation (Ibrahim and Mohamed, 2021). Squalene oxidation occurs earlier than other lipids, effectively interrupting peroxidative reactions by breaking lipid peroxidation chains. This mechanism helps stabilize and protect cell membranes (Micera *et al*., 2020).

In this line of research, *Cucurbita pepo* L. seed extract revealed the existence of various bioactive compounds possessing antioxidant, anti-inflammatory, and antimicrobial properties. These compounds hold potential for application in the development of safe functional health food products as well as in the formulation of pharmaceutical drugs.

PHASE III

4.3. In vitro Antioxidant Activity of *Cucurbita pepo* L. Seed Aqueous Extract

4.3.1. DPPH radical scavenging assay

The DPPH assay measures the concentration of antioxidants required to reduce the initial DPPH radical concentration by 50% (IC_{50}). The lowest IC_{50} values indicate the highest antioxidant capacity. This method is commonly used to assess the ability of plant extracts or compounds to function as free radical scavengers or hydrogen donors, and it is considered a rapid assay for evaluating the antioxidant potential of plant extracts (Gulcin and Alwasel, 2023). Table VI demonstrates the DPPH radical scavenging activity of *Cucurbita pepo* L. seed aqueous extract.

Table VI

DPPH Scavenging Activity of *Cucurbita pepo* L. Seed Extract

Sl. No	Concentration (µg/mL)	% inhibition	
		Standard (Ascorbic acid)	*C. pepo* seed extract
1	10	26.12±0.34	10.00±0.16
2	20	38.19±0.56	16.03±0.32
3	40	47.56±0.62	28.64±0.38
4	60	58.37±0.77	37.38±0.46
5	80	67.74±0.83	45.76±0.52
6	100	77.38±0.97	52.34±0.59
		IC_{50}= 46.94	**IC_{50}= 90.35**

In the present study, the half-maximal inhibitory concentration (IC_{50}) for *Cucurbita pepo* L. seed aqueous extract and the ascorbic acid (standard) were found to be 90.35µg/mL and 46.94µg/mL, respectively. The *Cucurbita pepo* L. seed extract exhibited significant antioxidant activity, as indicated by its lower IC_{50} value. From the investigation, it was evident that the *Cucurbita pepo* L. seed extract had a considerable DPPH radical scavenging activity comparable to that of the standard ascorbic acid. Furthermore, the inhibition of DPPH radical scavenging by the extract was strictly proportional to the concentration of total phenolics. These findings suggested that the high scavenging activity of the aqueous extract could be attributed to the presence of various bioactive compounds, including phenolics, flavonoids, and alkaloids, as confirmed by the qualitative and quantitative phytochemical screening. Hence the study strongly implies a significant correlation between the antioxidant activity and phenolic content of *Cucurbita pepo* L. seeds.

Valenzuela *et al*., (2014) reported that in all varieties of seed Cucurbita spp studied, the highest antiradical activity was detected in the aqueous fractions. A study carried out by Rakass *et al*., (2018) also disclosed that among all extracts, water extract of *Cucurbita pepo* L. exhibited the highest antioxidant activity, followed by ethanol, methanol, and acetone extracts. Oomah *et al*., (2011) reported in the study of phenolic and antioxidant activity of various extracts of lentil and pea hulls (acetone, ethanol, water and hot water extracts), that the most potent phenolics were estimated in water extract of red and green lentil hulls. Numerous studies have proven that antioxidant activity of plant extracts is mainly associated to the total phenolic content in the plants. In the present study, a strong correlation was observed between total phenolic, total flavonoid, and total alkaloid contents and the radical scavenging activity of *Cucurbita pepo* L. seed extract.

4.3.2. Hydrogen peroxide scavenging assay

Hydrogen peroxide is a harmful reactive species, if produced in excess in living organisms. It can easily penetrate cell membranes, once inside, transform into hydroxyl radicals causing significant cellular damage. Antioxidants counteract this damage by donating electrons to hydrogen peroxide radicals to stabilize them. The donated electron reacts with hydrogen peroxide, converting it into water and neutralizing the radical. The ability of the extract to quench hydrogen peroxide radicals determines the antioxidant potential of the extract (Ofoedu *et al.*, 2021). The hydrogen peroxide radical scavenging activity of the *Cucurbita pepo* L. seed aqueous extract was assessed and is represented in Table VII.

Table VII

Hydrogen Peroxide Scavenging Activity of *Cucurbita pepo* L. Seed Extract

Sl. No	Concentration (µg/mL)	Percentage inhibition	
		Standard (Ascorbic acid)	*C. pepo* seed extract
1.	100	39.12±0.16	23.29±0.11
2.	200	56.11±0.39	42.55±0.32
3.	300	77.19±0.21	64.17±0.26
4.	400	86.27±0.02	78.37±0.74
5.	500	94.14±0.56	84.54±0.03
		IC_{50}= 153.33	**IC_{50}= 245.81**

Though percentage inhibition of hydrogen peroxide radicals exhibited by *Cucurbita pepo* L. seed aqueous extract was lower than the inhibition expressed by the standard ascorbic acid, the study revealed that the extract presented a prominent antioxidant activity. The H_2O_2 scavenging activity of *Cucurbita pepo* L. seed aqueous extract was similar to the findings of Soni and Bali (2019), who also reported similar antioxidant effects. The assay revealed that the inhibition exhibited by the extract was concentration-dependent. The presence of phenolic compounds, which contain phenolic hydroxyl groups, is likely responsible for the extract’s notable antiradical properties. Compounds with antioxidant activity function as free radical scavengers, reducing agents, and quenchers of singlet-oxygen or Reactive Oxygen Species, thus providing protection against degenerative diseases and disorders (Mitra, 2020).

4.3.3. Superoxide radical scavenging assay

The superoxide radical scavenging activity of the *Cucurbita pepo* L. seed aqueous extract was analyzed and represented in Figure 5.

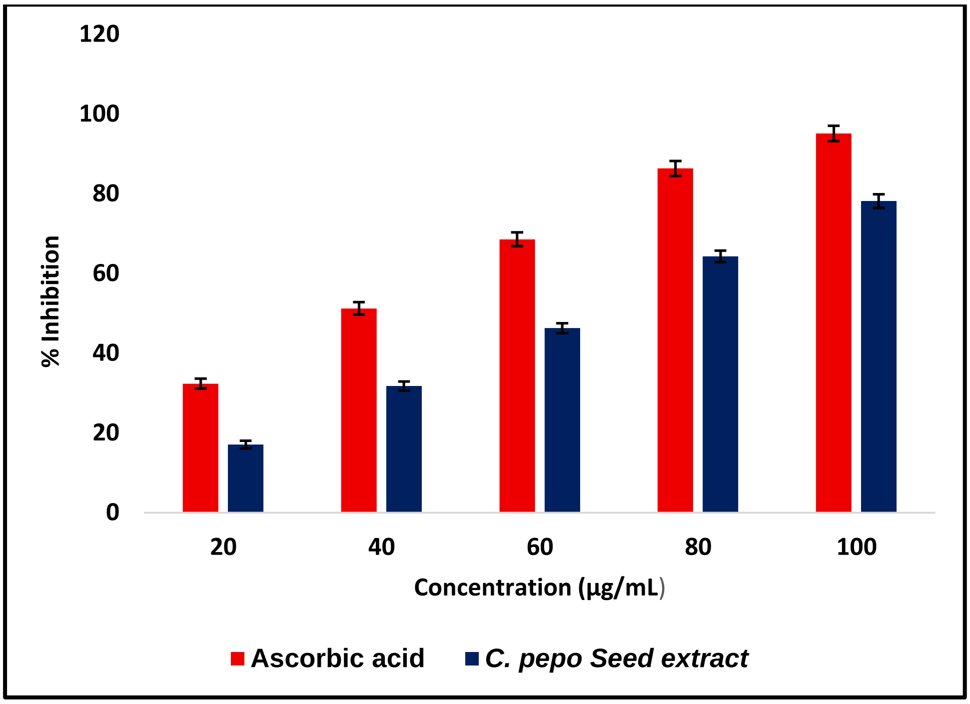

Figure 5: Superoxide Radical Scavenging Assay of *Cucurbita pepo* L. seed Extract

Percentage inhibition of superoxide radical scavenging by *Cucurbita pepo* L. seed aqueous extract was lower than that expressed by the standard ascorbic acid. Nevertheless, the study revealed that the *Cucurbita pepo* L. seed extract had a prominent antioxidant activity. The assay confirmed that the inhibition exhibited by the extract was in a concentration dependent manner. The presence of phenolic compounds (containing phenolic hydroxyls) found in the seed extract could be attributable to the observed antiradical properties.

4.3.4. Ferric Reducing Antioxidant Power (FRAP) assay

Aqueous extract of *Cucurbita pepo* L. seeds disclosed a good reducing powder activity that was comparable with ascorbic acid as given in Table VIII.

Table VIII

FRAP Assay of *Cucurbita pepo* L. Seed Extract

Sl. No	Concentration (µg/mL)	Optical Density	
		Standard (Ascorbic acid)	*C. pepo* seed extract
1.	20	0.43	0.11
2.	40	0.57	0.27
3.	60	0.72	0.42
4.	80	0.87	0.58
5.	100	0.99	0.69

The reductive ability is primarily attributed to the Fe^{3+} to Fe^{2+} transformation, which takes place in the presence of the sample extract. The presence of reductones is often linked to the antioxidant activity, as they can break free radical chains by donating a hydrogen atom. Additionally, reductones are known to react with precursors of peroxides, preventing peroxide formation. Increased absorbance of the reaction mixture indicates increased reducing powder (Sihag *et al*., 2022).

The reducing power of *Cucurbita pepo* L. seed aqueous extract was found to be remarkable, with its activity rising as the concentration of the extract was gradually raised. The study discovered that the *Cucurbita pepo* L. seed aqueous extract exhibited exceptional reducing activity. The FRAP assay further confirmed the concentration-dependent activity of the seed extract.

The presence of various bioactive compounds, phenolics in particular, could be responsible for the observed reducing power ensuring the sample extract possess high antioxidant potential and thereby of highly therapeutic significance. The results of this current study were in accordance with Ethiraj and Sridar (2018), who reported that plant phenolics constitute one of the major groups of compounds acting as primary antioxidant free radical terminators.

PHASE IV

4.4. Acute Toxicity study of *Cucurbita pepo* L. seed aqueous extract in adult female Wistar rats

Acute toxicity refers to the undesirable effects caused by a single exposure or multiple brief exposures to a toxic substance over a short span, typically within 24 hours. Acute toxicity is generally assessed within 14 days of administration of the substance (OECD, 2000). They are carried out to establish the median Lethal Dose (LD_{50}) of a substance (Saganuwan, 2017). The investigation was carried out to figure out the maximum tolerable dose to establish the safety of the *Cucurbita pepo* L. aqueous seed extract.

According to OECD (2000), female rats are the preferred rodent species for acute toxicity studies. Lipnick *et al.*, (1995) pointed out that studies of conventional LD_{50} tests exhibited a slight variation in sensitivity amongst male and female, in those cases variations are detected and female animals were usually extra sensitive than male.

Acute toxicity study was conducted on twelve female Wistar rats (nulliparous and non-pregnant), aged 12-14 weeks and weighing approximately 120 grams. The control group was administered distilled water, while the experimental groups received aqueous extract of *Cucurbita pepo* L. seeds at varying doses of 50 mg, 300 mg, and 2000 mg /kg body weight. The rats were observed for a period of 14 days, with particular attention paid to the first 30 minutes, four hours, and the following 24 hours after supplementation.

4.4.1. General observation of acute toxicity of *Cucurbita pepo* L. seed extract in adult female rats

Table IX represents the general observation of acute toxicity study of *Cucurbita pepo* L. seeds aqueous extract on adult female rats.

Table IX

General Observation of Acute Toxicity of *Cucurbita pepo* L. Seed Extract

General Observation	Control	EG I (50 mg/kg)	EG II (300 mg/kg)	EG III (2000 mg/kg)
Breathing	Normal	Normal	Normal	Normal
Food Intake	Normal	Normal	Normal	Normal
Changes in skin & fur	No changes	No changes	No changes	No changes
Body weight	Normal	Normal	Normal	Normal
Drowsiness	Absent	Absent	Absent	Absent
Sedation	Absent	Absent	Absent	Absent
Alive/Dead	**Alive**	**Alive**	**Alive**	**Alive**

Source: OECD 423

Control Group – distilled water

Experimental Groups (EG I, EG II, EG III) – *Cucurbita pepo* L. seed aqueous extract

After the administration of a single high dose of *Cucurbita pepo* L. seed aqueous extract (2000 mg/kg/b.w.), none of the animals in the experimental groups displayed signs of toxicity in their general behaviour. The rats showed normal body weight, regular breathing patterns, consistent food intake, and no noticeable changes in skin or fur. Furthermore, no toxic effects such as drowsiness, sedation, or coma were observed. By the end of the observation period, all the animals in the toxicity study group remained alive, indicating that the sample extract was safe for supplementation.

4.4.2. Observation of gross behaviour of acute toxicity of *Cucurbita pepo* L. seed extract in adult female rats

(i) Observation of Central Nervous System stimulant activities

Observation of Central Nervous System (CNS) stimulant activities in association with acute toxicity study are expressed in Table X.

Table X

Observation of Central Nervous System Stimulant Activities

Gross Behaviour	Control	EG I (50 mg/kg)	EG II (300 mg/kg)	EG III (2000 mg/kg)
Irritability	X	X	X	√
Hyperactivity	X	X	X	√
Convulsions	X	X	X	X
Analgesia	X	X	X	X
Stereotype	X	X	X	X
Tremor	X	X	X	X
Stub Tail	X	X	X	X

Source: OECD 423

√- Present

X- Absent

Control Group – distilled water

Experimental Groups (EG I, EG II, EG III) – *Cucurbita pepo* L. seed aqueous extract

Rats exhibited irritability and hyperactivity after receiving a high dose of *Cucurbita pepo* L. seed aqueous extract (2000 mg/kg body weight). These central nervous system stimulant effects, including irritability and hyperactivity, were observed immediately following supplementation and gradually subsided within 3-4 hours.

(ii) Observation of Central Nervous System (CNS) depression activities

Observation of Central Nervous System (CNS) depression activities in association with acute toxicity study are expressed in Table XI.

Table XI

Observation of Central Nervous System Depression Activities

General Observation	Control	EG I (50 mg/kg)	EG II (300 mg/kg)	EG III (2000 mg/kg)
Hypo activity	X	X	X	X
Passivity	X	X	X	X
Relaxation	X	X	X	X
Ataxia	X	X	X	X

Source: OECD 423

X- Absent

Control Group – distilled water

Experimental Groups (EG I, EG II, EG III) – *Cucurbita pepo* L. seed aqueous extract

The behaviour and locomotor activity of the animals in both the control and test groups remained unaltered. No signs of Central Nervous System (CNS) depression, such as hypoactivity, passivity, relaxation, or ataxia, were observed in the experimental groups following supplementation with *Cucurbita pepo* L. seed aqueous extract.

(iii) Observation of Autonomous Nervous System (ANS) activities

Activities of the Autonomous Nervous System (ANS) observed in association with acute toxicity study are expressed in Table XII.

Table XII

Observation of Autonomous Nervous System Activities

General Observation	Control	EG I (50 mg/kg)	EG II (300 mg/kg)	EG III (2000 mg/kg)
Salivation	X	X	X	√
Frequent urination	X	X	X	√
Ptosis	X	X	X	X
Exophthalmia	X	X	X	X
Lacrimation	X	X	X	X

Source: OECD 423

√- Present

X- Absent

Control Group – distilled water

Experimental Groups (EG I, EG II, EG III) – *Cucurbita pepo* L. seed aqueous extract

Autonomous Nervous System (ANS) activities such as frequent urination and salivation were observed in the group supplemented with *Cucurbita pepo* L. seed aqueous extract of a high dose of 2000 mg/kg body weight. However, these ANS activities got diminished within 3-4 hours following administration. Despite the occurrence of these transient effects, the high dose did not lead to significant toxicity or mortality in any of the test animals. In line with this, Chari *et al*., (2018) reported that pumpkin seed extract was safe at doses up to 2000 mg/kg body weight in Wistar rats, as no signs of toxicity or mortality were observed. Based on these findings, the study was continued using dosages within the established safe range (100 and 1000 mg/kg body weight).

PHASE V

4.5. Evaluation of the Antioxidant Potential of *Cucurbita pepo* L. Seed Aqueous Extract in the Treatment of Stress Induced Male Infertility

4.5.1. Determination of the effects of *Cucurbita pepo* L. seed extract on stress induced infertile male rats in association with physical parameters

A. Body weight assessment

The initial (before commencing the experiments) and final (after experimentation) body weights of the rats were assessed. Mean body weight gain of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is presented in Table XIII.

Table XIII

Mean Body Weight of Control and Treatment Groups

Groups N=6	Treatment	Average Body Weight (g)		N.W.G. (g)
		Day 1	**Day 45**	
CONTROL	Standard diet	139.7±0.954	171.8±2.227	32.1
LA	Lead acetate alone (30 mg/kg)	143.2±1.833	169±1.317	25.8
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	142.8±2.182	174.7±3.263	31.9
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	147.3±2.616	176.3±2.835	29
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	138.7±1.843	169.3±1.358	30.6

N.W.G. – Net Weight Gain

The experimental rats were monitored for changes in body weight from the start to the end of the experiment. All the rats under the study were of approximately of equal body weight (142±5 grams) initially. The control rats exhibited a steady weight gain throughout the experimental period. The severe effect on body weight was found in the lead acetate alone ingested rats (without the administration of *Cucurbita pepo* L. seed extract) clearly indicating that lead ingestion causes a significant decrease in body weight gain. This result was supported by Saeed (2017) whose findings reported that lead causes decrease in growth rate in rats when fed lead. The decline in body weight gain may be caused by the toxic ions which could be associated with imbalanced metabolism. *Cucurbita pepo* L. seed extract when treated alone (without lead administration) PSE rats showed a steady and remarkable weight gain as that of control. Nevertheless, LA+PSE LD and LA+PSE HD presented a significant dose dependent improvement in the weight gain which was affected upon lead administration. This result confirmed that the reversal effect of the *Cucurbita pepo* L. seed extract on the lead induced body weight reduction was in a concentration dependent manner.

Dietary inclusion of raw and roasted pumpkin seeds had apparent protective effects on body weight in comparison with cisplatin-induced nephrotoxic untreated rats ascribing to the improved antioxidant status as reported by Oyetayo *et al.*, (2020). Present study also highlights the presence of antioxidants which favours protein by depressing oxidative damage. The above finding revealed the anabolic effect of *Cucurbita pepo* L. seed extract against adverse effect of lead toxicity on body weight.

B. Organ weight assessment

i) Brain weight

Mean brain weight of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is presented in Table XIV.

Table XIV

Mean Brain Weight of Control and Treatment Groups

Groups	Treatment	Brain (g)
CONTROL	Standard diet	1.475±0.286
LA	Lead acetate alone (30 mg/kg)	1.635±0.107^{ns}
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	1.462±0.092^{ns}
LA+PSE LD	Lead acetate (30 mg/kg b.w.) + *C. pepo* seed extract low dosage (100 mg/kg)	1.628±0.055^{ns}
LA+PSE HD	Lead acetate (30 mg/kg b.w.) + *C. pepo* seed extract high dosage (1000 mg/kg)	1.522±0.064^{ns}

*******p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison test was performed. Treated groups were compared with control group.

The results displayed in Table XIV regarding the brain weight indicated that there was no significant difference noted among all the treated groups regardless of the compound and /dosage administered. This finding was contradictory to the study conducted by Khalaf *et al*., (2012) who examined neurotoxic effects of lead and reported that there was highly significant reduction in brain/ body weight ratio in lead exposed rats. Lee *et al*., (2002) found that, compared to the vehicle-treated control, there were no significant changes in body and brain weight in mice groups administered with either NMDA or vegetable extracts.

ii) Liver weight

Mean liver weight of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is presented in Table XV.

Table XV

Mean Liver Weight of Control and Treatment Groups

Groups	Treatment	Liver (g)
CONTROL	Standard diet	7.765±0.245
LA	Lead acetate alone (30 mg/kg)	10.683±0.693***
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	7.175±0.335^{ns}
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	8.815±0.205*
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	7.952±0.689^{ns}

****p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison test was performed. Treated groups were compared with control group.

Table XV depicted an increase in liver weight in lead acetate treated group when compared to that of control group. *Cucurbita pepo* L. seed aqueous extract at low and high dosages exhibited dose dependent reversal in liver weight compared to LA group. *Cucurbita pepo* L. seed aqueous extract alone treated (PSE) retained the liver weight similar to control.

Several reports showed that lead caused many forms of illness to human organs (Engwa *et al*., 2019). Busari *et al.*, (2021) investigated the effects of lead acetate exposure on male rats and stated that lead significantly increased liver weight, along with histopathological changes and biochemical markers of oxidative stress in the liver highlighting the hepatotoxicity of lead. In another study, Mehmood *et al*., (2024) investigated the potential hepatoprotective effects of pumpkin seed extract in poultry chicken exposed to lead acetate. The results showed a significant reversal of liver weight increase and a reduction in liver damage markers, suggesting that pumpkin seed extract can protect against lead-induced hepatotoxicity.

iii) Kidney weight

Mean kidney weight of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is furnished in Table XVI.

Table XVI

Mean Kidney Weight of Control and Treatment Groups

Groups	Treatment	Kidney (g)
CONTROL	Standard diet	2.112±0.012
LA	Lead acetate alone (30 mg/kg)	2.566±0.104**
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	1.985±0.095^{ns}
LA+PSE LD	Lead acetate (30 mg/kg b.w.) + *C. pepo* seed extract low dosage (100 mg/kg)	2.255±0.078^{ns}
LA+PSE HD	Lead acetate (30 mg/kg b.w.) + *C. pepo* seed extract high dosage (1000 mg/kg)	2.134±0.058^{ns}

****p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

Table XVI depicted a significant increase in kidney weight of LA group when compared to control group. Rest of the groups were found to have no statistical difference in mean kidney weight indicating the *Cucurbita pepo* L. seed aqueous extract's potency in reversing lead induced toxicity reflected in kidney weight increment.

In chronic intoxication, kidney is the primary site for the accumulation of lead and the most critical organ that concentrates toxic substances in humans and animals, because of its highly specialized cells and large blood flow (Kuhad *et al*., 2007). Lead exposure is known to cause oxidative stress and damage to kidney tissues, resulting in kidney dysfunction. According to Oyetayo *et al.*, (2020) pumpkin seed extract significantly reduced kidney weight gain (a marker of kidney damage), improved renal function, and reduced oxidative stress markers. In alignment with this, the present study indicated that treatment with *Cucurbita pepo* L. seed extract reversed kidney hypertrophy (weight gain) attributing to the antioxidants present in the seeds such as phenolic compounds and flavonoids.

iv) Testes weight

Mean testes weight of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is given in Table XVII.

Table XVII

Mean Testes Weight of Control and Treatment Groups

Groups	Treatment	Testes (g)
CONTROL	Standard diet	2.753±0.038
LA	Lead acetate alone (30 mg/kg)	2.103±0.089**
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	2.697±0.021 ^{ns}
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	2.344±0.043*
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	2.613±0.020^{ns}

******p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

The weight of the testes was significantly (p<0.01) decreased in lead acetate treated group LA when compared to control group. Changes in testes weights may reflect changes in seminiferous tubules or interstitial edema (Creasy, 2002). The loss of weight in the testes could be owing to a declined level of androgen, which was inadequate to increase the weight of the gonads and accessories (Sharma and Jacob, 2001). *Cucurbita pepo* L. seed aqueous extract alone treated group PSE retained the testis weight with no significant alteration. Co-treatment with *Cucurbita pepo* L. seed aqueous extract in low dosage (LA+PSE LD) improved the testis weight. LA+PSE HD retained the same weight when compared to the control group indicating the potency of the *Cucurbita pepo* L. seed extract in reversing the adverse effects of lead administration in testis weight reduction.

v) Caudal Epididymis weight

Mean caudal epididymis weight of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is furnished in Table XVIII.

Table XVIII

Mean Caudal Epididymis Weight of Control and Treatment Groups

Groups	Treatment	Caudal epididymis (g)
CONTROL	Standard diet	0.355±0.020
LA	Lead acetate alone (30 mg/kg)	0.342 ± 0.017^{ns}
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	0.352 ± 0.003^{ns}
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	0.346 ± 0.012^{ns}
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	0.349 ± 0.003^{ns}

******p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

Table XVIII demonstrated no significant difference in caudal epididymis weight of all the treated groups to the control group. In contrast to the existing studies (Adamkovicova *et al*., 2013; Anjum *et al*., 2017; Maghraoui *et al*., 2023) on the effect of heavy metals on the weight of caudal epididymis, the current study found no significant change despite alterations in sperm quality and testicular histology, suggesting that lead toxicity does not always correlate with contraction in this region. The current study also highlights the potential of *Cucurbita pepo* L. seed extract in persevering the sperm quality without directly affecting epididymal weight.

vi) Seminal vesicle weight

Mean seminal vesicle weight gain of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is depicted in Table XIX.

Table XIX

Mean Seminal Vesicle Weight of Control and Treatment Groups

Groups	Treatment	Seminal vesicle (g)
CONTROL	Standard diet	0.633±0.006
LA	Lead acetate alone (30 mg/kg)	0.435±0.017***
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	0.619 ± 0.014^{ns}
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	0.527±0.248**
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	0.602 ± 0.237^{ns}

*******p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

Table XIX revealed significant diminution in seminal vesicle weight of LA group compared to the control group. *Cucurbita pepo* L. seed aqueous extract alone treated group PSE retained the seminal vesicle weight with no significant alteration. Co-treatment with *Cucurbita pepo* L. seed aqueous extract in low dosage (LA+PSE LD) improved the seminal vesicle weight. LA+PSE HD retained the same weight when compared to the control group indicating the potency of the sample extract in reversing the adverse effects of lead administration in seminal vesicle weight reduction.

vii) Prostate weight

Mean prostate weight gain of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is presented in Table XX.

Table XX

Mean Prostate Weight of Control and Treatment Groups

Groups	Treatment	Prostate (g)
CONTROL	Standard diet	0.538±0.023
LA	Lead acetate alone (30 mg/kg)	0.421±0.017**
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	0.525 ± 0.009^{ns}
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	0.498 ± 0.023^{ns}
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	0.514 ± 0.006^{ns}

******p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

Table XX revealed significant decline in prostate weight of LA group compared to the control group. Prostate weights may be associated with compound-related effects arising from modulation of androgenic or estrogenic signalling (Creasy, 2002). *Cucurbita pepo* L. seed aqueous extract alone and co-treated in low and high dosages retained the prostate weight with no significant alteration indicating the potency of the *Cucurbita pepo* L. seed extract in reversing the adverse effects of lead administration in prostate weight reduction.

4.5.2. Determination of the effects of *Cucurbita pepo* L. seed extract on stress induced infertile male rats in association with sperm parameters

Semen analysis is the most crucial test for determining whether a male is infertile. In fact, it continues to be the primary method for identifying male infertility. Further, sperm count, pH, motility, viability, and morphological abnormalities have all been connected to male factor infertility (Boitrelle *et al*., 2021).

i) Sperm Count

Spermatogenesis, a multi-step process where spermatozoa are produced by the germ cells inside the seminiferous tubules, has become crucial for male fertility. Spermatozoa concentration in the caudal region of the epididymis was measured immediately after sacrificing the experimental rats (Vyas and Raval, 2016). The sperm count of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is given in Figure 6.

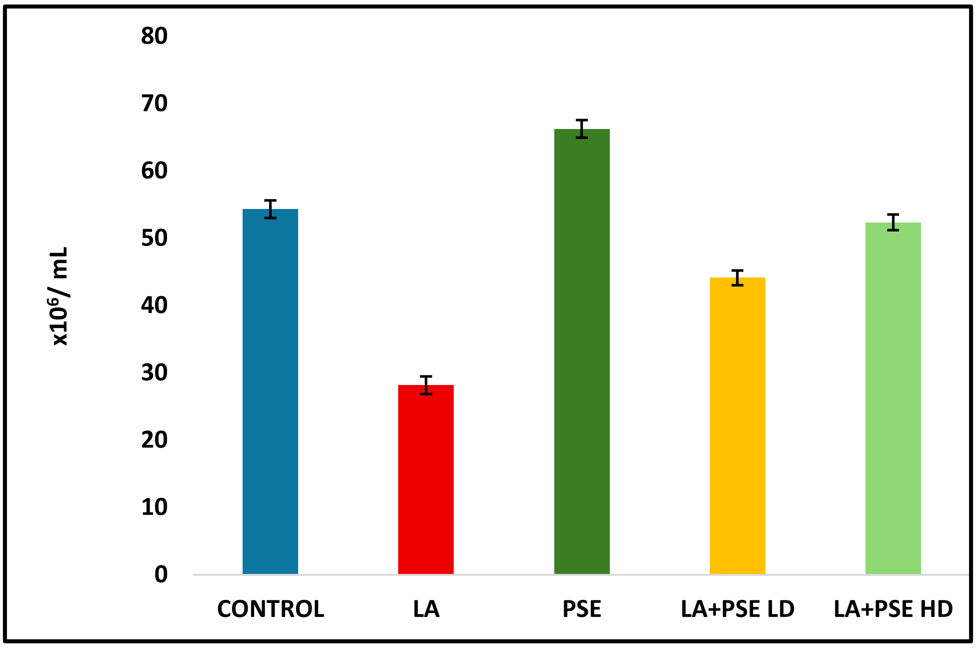

Figure 6: Sperm Count of Control and Treatment Groups

Control: Standard diet

LA: Lead Acetate alone

PSE: Pepo Seed Extract alone

LA+ PSE L.D: Lead acetate+ Pepo Seed Extract Low Dosage

LA+ PSE H.D: Lead acetate+ Pepo Seed Extract High Dosage

The epididymal sperm count results demonstrated that LA group showed severe lead toxicity induced oxidative stress as evidenced by a significant reduction of total number of sperms when compared to the control. Findings also confirmed that *Cucurbita pepo* L. seed extract treatment (PSE) had a highly significant improvement in sperm count when compared to control which indicated the potency of the seed extract in improving the reproductive potential. The co-treatment with *Cucurbita pepo* L. seed extract dose dependently mitigated the lead toxicity by improving the sperm count as evidenced by LA+ PSE LD and LA+ PSE HD groups.

This study concluded that *Cucurbita pepo* L. seed extract had a dose dependent reversal effect on the damage induced by lead exposure on epididymal sperm count. Shahin *et al*., (2021) found that antioxidants such as Vitamin C, Vitamin E, selenium, and zinc significantly improved sperm count, motility, and morphology. Vitamin C was better at improving reproductive dysfunction in male animals exposed to lead accumulation (Zhao *et al.,* 2023). These researchers concluded that antioxidants could be a beneficial adjunct to the treatment of male infertility, particularly in cases linked to oxidative stress.

ii) Sperm Motility

Sperm motility is the ability of sperms to move or swim efficiently through the female reproductive system in order to fertilize an egg. Motility issues, such as slow or zero progression, may negatively impact fertility. Hence sperm motility is an important aspect in fertility assessment (Dcunha *et al*., 2022). Sperm motility of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is discussed in Figure 7 as follows.

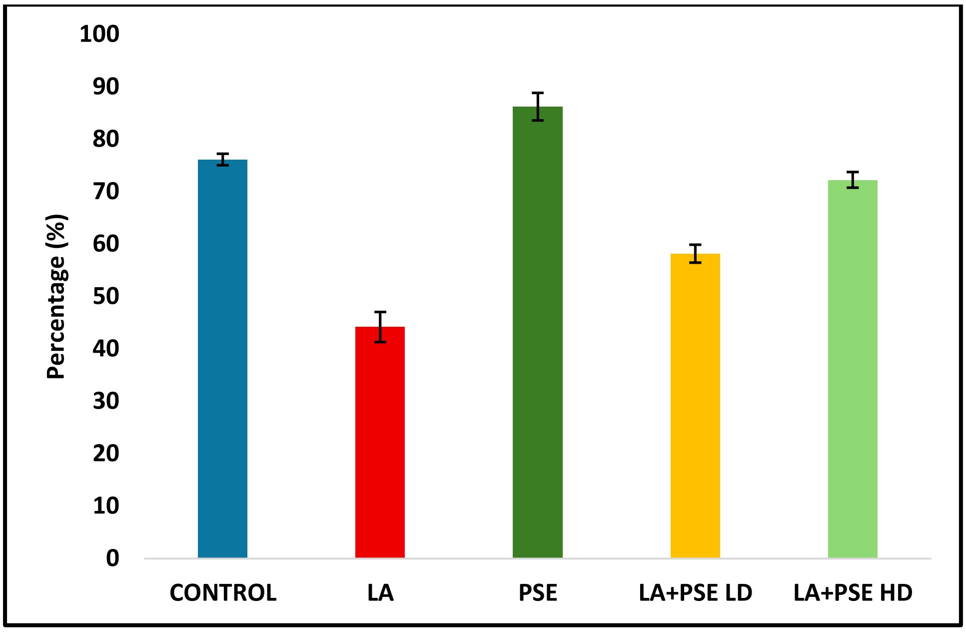

Figure 7: Sperm Motility of Control and Treatment Groups

Control: Standard diet

LA: Lead Acetate alone

PSE: Pepo Seed Extract alone

LA+ PSE LD: Lead acetate+ Pepo Seed Extract Low Dosage

LA+ PSE HD: Lead acetate+ Pepo Seed Extract High Dosage

Results indicated that there was a remarkable decline of motile sperms in the lead acetate treated alone LA group. The co-treatment with *Cucurbita pepo* L. seed extract recovered the immobility of sperms in a dose dependent manner implying the efficacy of the seed extract in reducing the pathogenesis induced by lead acetate ingestion. A similar effect was observed by Sudjarwo and Sudjarwo, (2017) and Assi *et al*., (2017) who reported a dose dependent protective effect of curcumin (active component of turmeric) and *Nigella sativa* on sperm motility against the lead acetate testicular toxicity respectively. Interestingly, *Cucurbita pepo* L. seed extract alone treated group PSE revealed a significantly higher percentage of motile sperms surpassing that of control group.

Agnihotri *et al*., (2016) highlighted sperm motility as a key determinant of male fertility. Carola (2020) demonstrated that metal compounds can negatively impact sperm quality by inhibiting sperm motility and altering the morphology of the sperm head membrane. Several studies have reported a strong correlation between antioxidant supplementation and male fertility exploring various therapeutic interventions (Dimitriadis *et al.*, 2023; Walke *et al.*, 2023; Abou-Shehema and Shahba, 2023). The present study is in alignment with the existing studies indicating the efficacy of natural antioxidant source like *Cucurbita pepo* L. seed extract in mitigating heavy metal toxicity and improving sperm motility, a vital sperm characteristic of male fertility.

iii) Sperm Viability

Sperm viability is defined as the percentage of live sperms found in a semen sample. Many non-moving sperms are alive, but unable to swim due to a biochemical or structural problem (Laoung-On *et al.*, 2021). Sperm viability of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is presented in Figure 8. Microscopic images of sperm viability are shown in **Plate 5**.

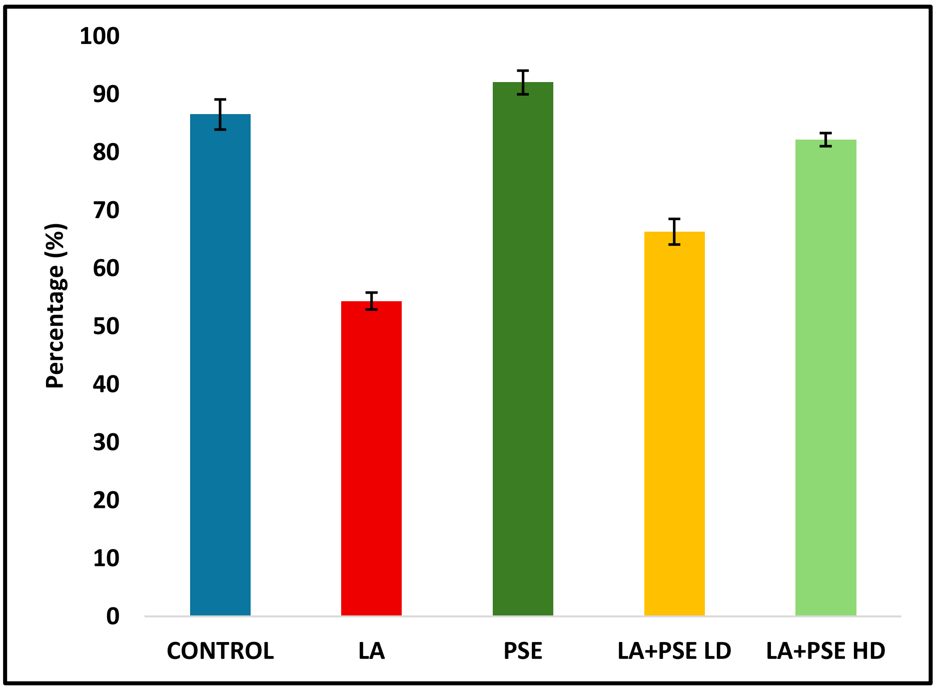

Figure 8: Sperm Viability of Control and Treatment Groups

Control: Standard diet

LA: Lead Acetate alone

PSE: Pepo Seed Extract alone

LA+ PSE L.D: Lead acetate+ Pepo Seed Extract Low Dosage

LA+ PSE H.D: Lead acetate+ Pepo Seed Extract High Dosage

Sperm viability results revealed that in comparison to control group, lead acetate treated alone group (LA) exhibited a highly significant ($p<0.001$) reduction in sperm viability percentage. Administration of *Cucurbita pepo* L. seed extract alone (PSE) indicated a significant ($p<0.05$) increment in sperm viability when compared to control group. *Cucurbita pepo* L. seed extract co-treatment at low dosage (LA+PSE LD) demonstrated significant ($p<0.01$) improvement when compared to LA group whereas a dose dependent improvement was achieved by high dosage (LA+PSE HD) group almost comparable to that of control.

A study conducted by Wahab *et al*., (2019) reported that there was a significant reduction ($p<0.05$) in sperm viability when the experimental rats were treated with lead acetate when compared with controls. Khalil *et al.,* (2024) demonstrated that quinoa seed extract supplementation following cryopreservation possessed beneficial effect on ram sperm viability. The natural antioxidants such as phenolic compounds and flavonoids alone or synergistically act as metal chelators and thereby reduces oxidative stress. The current study is an indicator of the robust antioxidant capacity of *Cucurbita pepo* L. seed extract as evidenced by improved percentage of sperm viability.

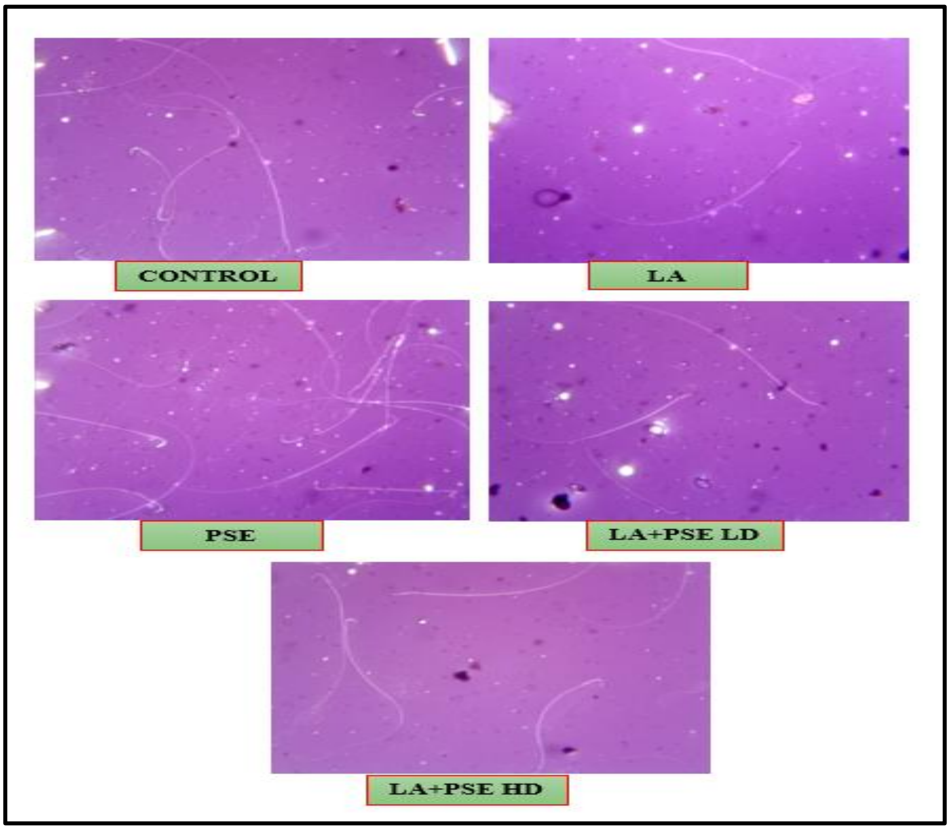

Plate 5: Sperm Viability of Experimental Rats

iv) Sperm Abnormality

Sperm morphology assessment, conducted through a stained semen smear, reveals a wide range of morphological variations or abnormalities in the sperm head, midpiece, and tail. According to Gatimel *et al*., (2017), sperm morphology serves as a reliable indicator of the health of the germinal epithelium. The sperm abnormality of the control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is as shown in Table XXI. Morphology of normal and abnormal sperms are shown in **Plate 6**.

Table XXI

Sperm Abnormality of Control and Treatment Groups

Groups	Treatment	Sperm Abnormality (%)
CONTROL	Standard diet	6.86±0.0577
LA	Lead acetate alone (30 mg/kg)	27.45±0.086***
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	4.32±0.202*
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	14.67±0.116***
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	9.58±0.0577*

****p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

From the present study, it could be deduced that when compared to control group, lead acetate treated alone group (LA) had a remarkable increment in the percentage of total sperm morphological anomalies. There was dose dependent decrement observed upon the co-administration of *Cucurbita pepo* L. seed extract as evidenced in LA+PSE LD & LA+ PSE HD groups. *Cucurbita pepo* L. seed extract alone treated group PSE revealed a lower percentage of abnormal sperms when compared to control group indicating the potency of the seeds in improving sperm quality.

Aldaddou *et al.,* (2022) proposed the ameliorative effect of methanolic extract of *Tribulus terrestris* L. on nicotine and lead-induced degeneration of sperm quality by improving sperm morphology. The current study is in accordance with the above findings indicating the mitigating effect of *Cucurbita pepo* L. seed extract against lead toxicity induced deterioration in sperm quality. This finding could be an insight into the powerful antioxidants present in the *Cucurbita pepo* L. seed extract which boosts the fertility potential by counteracting the adverse effects of lead exposure.

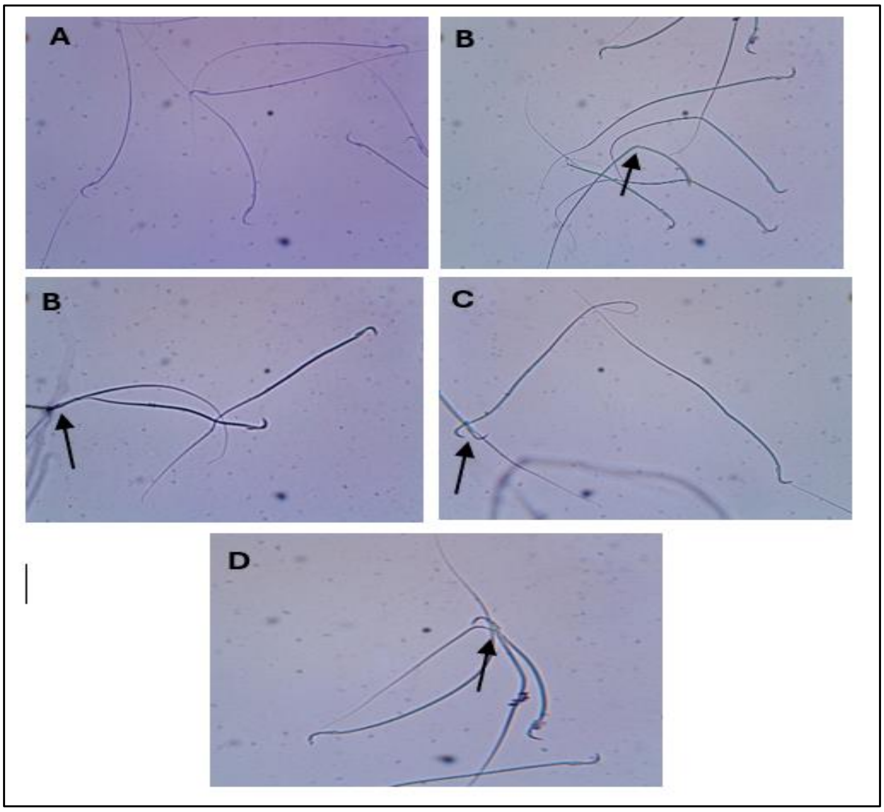

Plate 6: Morphology of Normal and Abnormal Sperms

A: Normal morphology; B: bent tail; C: double head; D: bent neck

v) Semen pH

The microenvironment of sperm, particularly the seminal plasma, plays a crucial role in sperm function. Seminal plasma is a complex mixture of secretions from the testes, epididymides, and accessory sex glands. It contains various components, including HCO3-/CO2, inorganic ions, organic acids, sugars, lipids, steroids, amino acids, polyamines, nitrogenous bases, and proteins. As a result, semen has an exceptionally high buffering capacity, surpassing that of most other body fluids. This buffering capacity suggests that the pH of seminal fluid is essential not only for maintaining sperm viability and quality but also for facilitating successful fertilization (Zhou *et al*., 2015). Semen pH of the control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation was analyzed and results are as furnished in Figure 9.

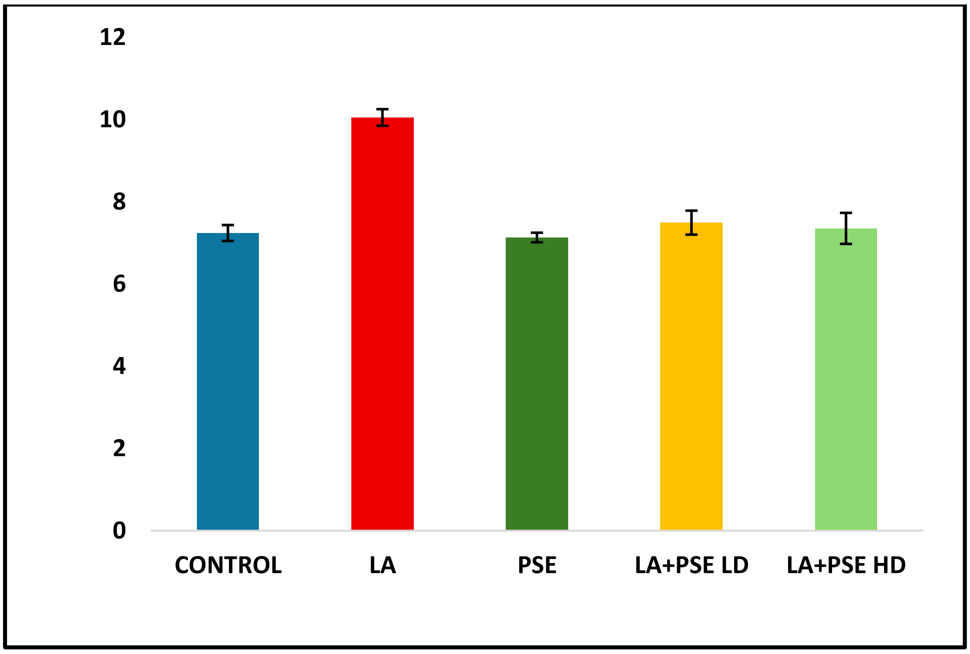

Figure 9: Semen pH of Control and Treatment Groups

Control: Standard diet

LA: Lead Acetate alone

PSE: Pepo Seed Extract alone

LA+ PSE L.D: Lead acetate+ Pepo Seed Extract Low Dosage

LA+ PSE H.D: Lead acetate+ Pepo Seed Extract High Dosage

Semen pH results revealed that lead acetate alone treated group (LA) exhibited a highly significant increment in the pH making it highly alkaline in nature. An imbalanced pH can hinder their journey towards the egg, affecting the chances of successful conception implying conditions such as hypospermia or azoospermia. On the other hand, *Cucurbita pepo* L. seed extract maintained a neutral pH (ideally 7.2-8) which is desirable for an effective sperm health. These results are suggestive of how the antioxidant potential of *Cucurbita pepo* L. seed mitigates the declined sperm quality which is the primary reason for male infertility.

4.5.3. Determination of the effects of *Cucurbita pepo* L. seed extract on stress induced infertile male rats in association with reproductive hormones

As much as female hormones are vital for fertility, male hormones are equally important. Hormones such as Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), and testosterone are imperative for procreation (Oduwole *et al*., 2021).

(i) Serum Luteinizing Hormone (LH)

Gonadotropin-releasing hormone (GnRH) stimulates the release of Luteinizing Hormone (LH), which then prompts the cells in the testes to produce testosterone. A low level of LH can lead to a reduced sex drive and fertility problems, while an elevated LH level can cause testicular damage (Oduwole *et al*., 2021). Serum LH level of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation are as follows in Table XXII.

Table XXII

Serum LH of Control and Treatment Groups

Groups	Treatment	Serum LH (IU/L)
CONTROL	Standard diet	2.147±0.462
LA	Lead acetate alone (30 mg/kg)	1.104±0.428***
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	2.089±0.257^{ns}
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	1.569±0.642**
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	1.998±0.303*

****p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

Table XXVI revealed that LA group exhibited a highly significant reduction in Luteinizing hormone when compared to control group. On the contrary, *Cucurbita pepo* L. seed extract treated alone PSE group retained the serum LH level intact similar to control group indicating no significant alteration. However, co-administration of seed extract concentration dependently improved the serum LH levels.

In their research, Al-Masri, (2015) observed that treatment with either or both of vitamin E and pumpkin seed oil substantially amended the lead induced anomalies by significant improving serum LH, FSH and testosterone hormone levels. Ekeleme-Egedigwe *et al*., (2019) discovered that the garlic oil (rich in essential bioactive compounds) supplemented diet significantly (p<0.05) improved serum LH, FSH and testosterone levels when compared to control group. Another antioxidant rich source, *Moringa oleifera* supplements in bucks' diets boosted serum LH as observed by Jimoh *et al*., (2021).

This could be due to the bioactive components such as phytosterols which have been shown to influence hormone metabolism and the abundance of phenolics and flavonoids.

(ii) Serum Follicle Stimulating Hormone (FSH)

Follicle Stimulating Hormone (FSH) plays a vital role in both male and female fertility. In males, FSH plays a vital role in testicular growth, as well as the production and maturation of sperm cells. Like LH, low levels of FSH can result in reduced libido or difficulty in conceiving, while elevated FSH levels may indicate testicular damage (Recchia *et al*., 2021). Serum FSH level of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation were assessed and interpreted as follows in Table XXIII.

Table XXIII

Serum FSH of Control and Treatment Groups

Groups	Treatment	Serum FSH (IU/L)
CONTROL	Standard diet	1.817±0.549
LA	Lead acetate alone (30 mg/kg)	0.803±0.324***
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	1.729±0.638^{ns}
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	1.113±0.224**
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	1.256±0.542**

****p<0.001, **p<0.01, *p<0.05, ns-not significant*

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett’s comparison was performed. Treated groups were compared with control group.

Serum FSH results demonstrated that LA group exhibited a highly significant ($p<0.001$) reduction when compared to control group. *Cucurbita pepo* L. seed extract treated alone PSE group retained the serum FSH level intact similar to the control group which proved that the seed extract has a potency to retain and maintain the normal FSH level. Interestingly, co-administration of seed extract concentration dependently improved the serum FSH levels.

Oyeyemi *et al*., (2020) observed that oral lead exposure to rats at dose of 10 mg/kg body weight showed a significant reduction in the level of LH and FSH when compared to the control group. Significant degrees of improvement ($P\leq0.05$) were noted in male reproductive hormones concentration along with increased doses of pumpkin seed oil compared with chlorpyrifos treated animals (Abed and Alkalby, 2018). The present study revealed a similar effect on serum FSH level indicating the potency of *Cucurbita pepo* L. seed extract in regulating reproductive hormone levels.

(iii) Serum testosterone

Testosterone is produced through steroidogenesis, a process by which testes use cholesterol as a precursor. Testosterone is essential for a number of physiological and reproductive functions in males, and the testes are the main organelles that may produce it. Testosterone is thought to be indispensable for the development of secondary sexual characteristics in males because it induces libido, stimulates spermatogenesis, and promotes spermatogenesis (Patil *et al*., 2023). Serum testosterone level of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation were assessed and results are represented in Figure 10.

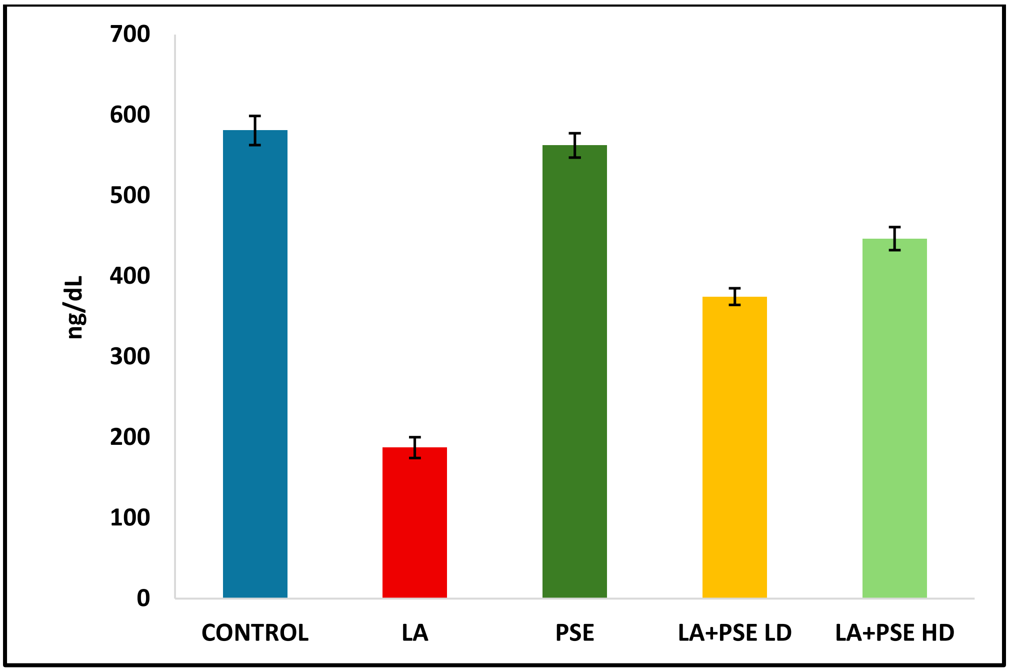

Figure 10: Serum Testosterone of Control and Treatment Groups

Control: Standard diet

LA: Lead Acetate alone

PSE: Pepo Seed Extract alone

LA+ PSE L.D: Lead acetate+ Pepo Seed Extract Low Dosage

LA+ PSE H.D: Lead acetate+ Pepo Seed Extract High Dosage

Figure 10 demonstrated that LA group exhibited a highly significant ($p<0.001$) reduction in serum testosterone level in comparison with the control group. *Cucurbita pepo* L. seed extract treated alone PSE group retained the testosterone same as control group whereas co-treatment with seed extract at both low and high dosages concentration dependently improved the testosterone level as evidenced by LA+PSE LD and LA+PSE HD groups.

Testosterone is essential for the development and maturation of sperm (Patil *et al.*, 2023). The diminished testosterone level in lead acetate treated rats in the current study is in agreement with the findings of Bidanchi *et al.*, (2022) who suggested that treatment with lead acetate significantly reduced sperm quality in addition to degenerative changes in seminiferous tubules and Leydig cells. This might be due to a combination of direct damage to the testes, induction of oxidative stress in Leydig cells, disruption of the Hypothalamic-

Pituitary-Gonadal (HPG) axis, and endocrine imbalances. The exact mechanisms may involve both direct and indirect effects on the organs and pathways involved in testosterone synthesis and regulation.

The study recommends that *Cucurbita pepo* L. seed extract could regulate testosterone biosynthesis and the raised testosterone level might be attributed to the potent bioactive compounds with antioxidant properties such as phytosterols and the abundant presence of minerals like zinc which is associated with testosterone production.

4.5.4. Determination of the effects of *Cucurbita pepo* L. seed extract on stress induced infertile male rats in association with antioxidant status

The balance between Reactive Oxygen Species (ROS) and antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), is essential for protecting cells from oxidative stress. This equilibrium plays a critical role in preventing damage caused by the harmful effects of ROS (Soliman *et al*., 2019). Effect of supplementation of *Cucurbita pepo* L. seed aqueous extract on antioxidant status in stress induced infertile male Wistar rats were assessed and interpreted as follows.

i) Serum Superoxide Dismutase (SOD)

Superoxide Dismutase (SOD) is an antioxidant enzyme that acts to degrade superoxide, a major causative factor for oxidative stress, an underlying factor associated with various ailments (Gusti *et al*., 2021). Serum SOD level of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation were assessed and results are represented in Table XXIV.

Table XXIV

Serum SOD of Control and Treatment Groups

Groups	Treatment	Serum SOD (unit/mg/min)
CONTROL	Standard diet	2.14±0.112
LA	Lead acetate alone (30 mg/kg)	0.92±0.015***
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	2.98±0.124**
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	1.57±0.153**
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	$1.98{\pm}0.125^{ns}$

****p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

Results disclosed a highly significant reduction in SOD levels in the lead acetate alone treated LA group indicating the high oxidative stress induced by lead. Co-administration of *Cucurbita pepo* L. seed extract improved the SOD level in a dose dependent manner. *Cucurbita pepo* L. seed extract when administered alone (PSE) showed a highly significant improvement in SOD level when compared to control highlighting the antioxidant potential of the extract.

Fipronil, a highly effective insecticide when exposed repeatedly to male albino mice, significantly decreased serum SOD (Abouelghar *et al*., 2020). Rouag *et al*., (2020) reported that pumpkin seed oil boosted the antioxidant status by significantly improving the serum SOD level in sodium nitrate exposed rats. The current study is in alignment with the above finding laying concrete evidence for the antioxidant potential of *Cucurbita pepo* L. seeds.

ii) Serum Glutathione Peroxidase (GPx)

Glutathione peroxidase is a family of enzymes with peroxidase activity, and its primary function is to protect the organism from oxidative damage. Biochemically, glutathione peroxidase reduces lipid hydroperoxides to their corresponding alcohols and converts free hydrogen peroxide into water, thereby alleviating oxidative stress (Handy and Loscalzo, 2022). Serum GPx level of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation were evaluated and results are presented as follows in Table XXV.

Table XXV

Serum GPx of Control and Treatment Groups

Groups	Treatment	Serum GPx (U/mg/min)
CONTROL	Standard diet	25.87±1.652
LA	Lead acetate alone (30 mg/kg)	13.92±1.173***
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	32.25±1.192**
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	18.05±1.032**
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	24.91±1.094*

****p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

The study indicated that the activity of the enzyme Glutathione peroxidase (GPx) was found to be significantly decreased in lead acetate alone treated group LA in comparison to control group. When the rats were co-administered with *Cucurbita pepo* L. seed aqueous extract, the enzyme activity was significantly improved in a concentration dependent manner.

The enhancement in the enzyme activity was maximum in the rats treated with *Cucurbita pepo* L. seed aqueous extract alone (PSE).

Abou-Shehema and Shahba, (2023) evaluated the effect of pumpkin seed powder supplementation in the diet of rabbit bucks and found that they significantly improved serum GPx compared to other experimental groups. Therefore, the present study confirms the enormous antioxidant capacity of *Cucurbita pepo* L. seeds.

iii) Serum Catalase

Catalase is a widespread enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the breakdown of hydrogen peroxide into water and oxygen. This enzyme plays a crucial role in protecting cells from oxidative damage caused by Reactive Oxygen Species (Hadwan *et al*., 2024). Serum catalase level of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation are represented in Table XXVI.

Table XXVI

Serum Catalase of Control and Treatment Groups

Groups	Treatment	Serum Catalase (µmole/mL/min)
CONTROL	Standard diet	0.38±0.013
LA	Lead acetate alone (30 mg/kg)	0.16±0.032***
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	0.59±0.068***
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	0.27±0.020**
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	$0.36±0.059^{ns}$

*******p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

The study indicated that serum catalase was significantly decreased ($p<0.001$) in lead acetate treated group LA in comparison to control group. Nonetheless, *Cucurbita pepo* L. seed aqueous extract treated alone (PSE) exhibited highly significant ($p<0.001$) improvement in catalase when compared to control group. *Cucurbita pepo* L. seed aqueous extract dose dependently indicated a significantly improved catalase activity at both low and high dosages.

Pumpkin seed extract enhanced serum indicators and normalized serum catalase levels as per the study by Lateef *et al.*, (2024) in rats with streptozotocin induced diabetes. The improved catalase activity observed in *Cucurbita pepo* L. seed extract treated rats could be owing to the potent antioxidants present in it.

iv) Serum Lipid Peroxidation (LPO)

Free radicals initiate the process of lipid peroxidation in an organism. Malondialdehyde (MDA) is one of the final products formed during the peroxidation of polyunsaturated fatty acids in cells. An increase in free radicals leads to an overproduction of MDA. As a result, the level of malondialdehyde is commonly used as a marker of oxidative stress (Gianazza *et al.*, 2021). Serum LPO of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation are demonstrated in Table XXVII.

Table XXVII

Serum LPO of Control and Treatment Groups

Groups	Treatment	Serum LPO (nmol/mg)
CONTROL	Standard diet	2.15±0.285
LA	Lead acetate alone (30 mg/kg)	3.82±0.128***
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	1.08±0.204**
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	2.94±0.159*
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	2.26±0.194^{ns}

****p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

The study indicated that serum lipid peroxidation level was significantly increased (p<0.001) in lead acetate treated group LA in comparison to control group. *Cucurbita pepo* L. seed aqueous extract treated alone (PSE) exhibited a highly significant (p<0.001) decrease in lipid peroxidation compared to control group. On the other hand, lead acetate co-treated with *Cucurbita pepo* L. seed aqueous extract at low and high dosages displayed a significant decrease in lipid peroxidation in a dose dependent manner. Hence, the *Cucurbita pepo* L. seed aqueous extract revealed a potent antioxidant activity against the lead induced oxidative stress.

Consistent with the present study, Mohammad *et al.*, (2021) found that pumpkin seed extract consumption in rats exposed to H_2O_2-induced oxidative damage led to a reduction in lipid peroxidation levels in heart tissue. Their study also noted that the effects of pumpkin seed consumption were dose-dependent.

v) Testis Superoxide Dismutase (SOD)

Results of testis SOD level of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation are given in following Table XXVIII.

Table XXVIII

Testis SOD of Control and Treatment Groups

Groups	Treatment	Testis SOD (unit/mg/min)
CONTROL	Standard diet	5.552±0.092
LA	Lead acetate alone (30 mg/kg)	2.435±0.012***
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	6.874±0.072**
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	4.259±0.016**
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	5.103±0.014^{ns}

*******p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

In comparison to the control group, lead acetate treated alone group LA significantly decreased (p<0.001) the superoxide dismutase level in the testis. Interestingly, *Cucurbita pepo* L. seed aqueous extract treated alone PSE presented a significant (p<0.01) increase in the testis superoxide dismutase level in comparison to the control group. *Cucurbita pepo* L. seed aqueous extract treated at low dosage showed a statistically significant (p<0.01) increase in the activity when compared to control group. In addition, *Cucurbita pepo* L. seed aqueous extract treated at high dosage expressed a similar SOD level compared to that of control group.

According to Reddy *et al.*, (2016), a high dose of curcumin (active component with antioxidant property) (200 mg/kg) potentially amplified the antioxidant enzyme activity significantly ($p<0.01$) and reduced lipid peroxidation level ($p<0.001$) when compared to the control group rats. This implies that treatment with antioxidant sources like *Cucurbita pepo* L. seed extract could reverse the toxicity induced by lead acetate evidenced by a significant increase in superoxide dismutase level in the testis homogenate.

vi) Testis Catalase (CAT)

Testis catalase level of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is represented in Figure 11.

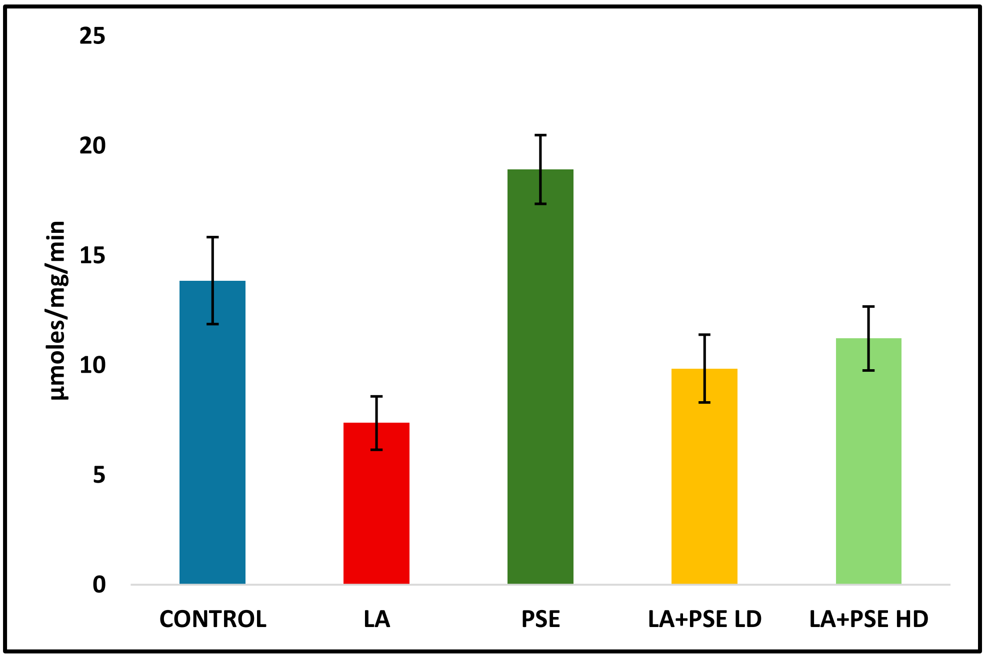

Figure 11: Testis CAT of Control and Treatment Groups

Control: Standard diet

LA: Lead Acetate alone

PSE: Pepo Seed Extract alone

LA+ PSE L.D: Lead acetate+ Pepo Seed Extract Low Dosage

LA+ PSE H.D: Lead acetate+ Pepo Seed Extract High Dosage

The study indicated that catalase activity in the testis homogenate was significantly decreased ($p<0.001$) in lead acetate treated group LA in comparison to control group. Nonetheless, *Cucurbita pepo* L. seed aqueous extract treated alone (PSE) exhibited highly significant ($p<0.001$) increase in catalase activity surpassing that of control group. *Cucurbita pepo* L. seed aqueous extract at low and high dosages indicated a significant improvement in catalase activity proving the dose dependent effect on reversing the lead toxicity effect on catalase activity.

Ghanbari *et al*., (2016) investigated the antioxidant effects of royal jelly by assessing the catalase activity in the testes of streptozotocin (STZ)-induced diabetic rats. Their results indicated that royal jelly significantly improved catalase levels in testicular tissues. Similarly, Hasona (2018) explored the ameliorative effects of grape seed extract in dexamethasone-induced testicular and thyroid dysfunction associated with oxidative stress, observing a significant increase in testicular catalase activity. These findings align with the current study, which demonstrates the antioxidant actions of *Cucurbita pepo* L. seed extract in protecting against oxidative damage caused by lead exposure.

vii) Testis Glutathione peroxidase (GPx)

Testis Glutathione peroxidase level of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is represented in Figure 12.

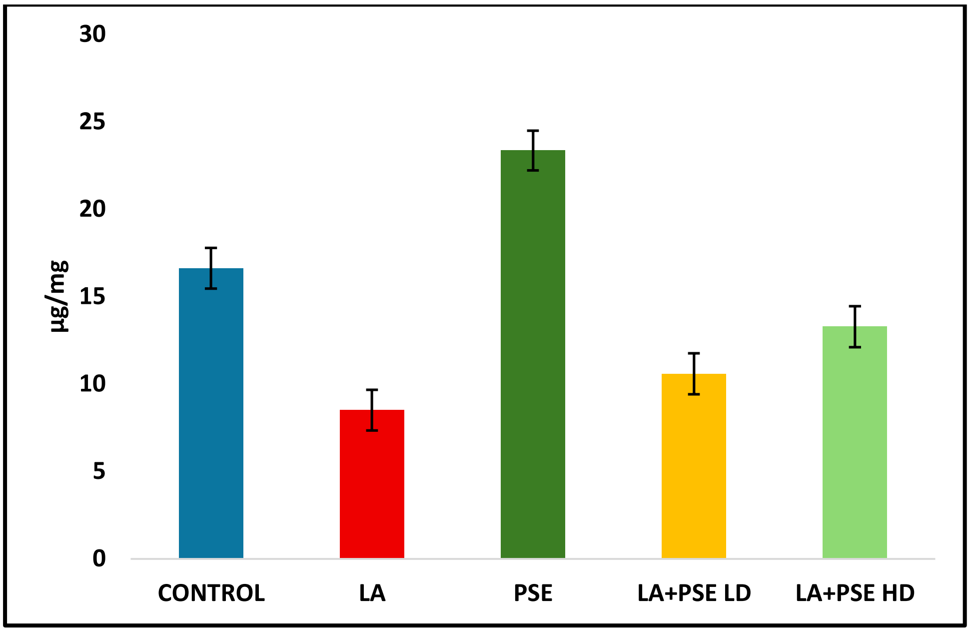

Figure 12: Testis GPx of Control and Treatment Groups

Control: Standard diet

LA: Lead Acetate alone

PSE: Pepo Seed Extract alone

LA+ PSE L.D: Lead acetate+ Pepo Seed Extract Low Dosage

LA+ PSE H.D: Lead acetate+ Pepo Seed Extract High Dosage

The study indicated that GPx levels were significantly decreased ($p<0.001$) in lead acetate treated group LA in comparison to control group. *Cucurbita pepo* L. seed aqueous extract treated alone exhibited GPx level significantly ($p<0.001$) higher than control group. *Cucurbita pepo* L. seed aqueous extract low dosage and high dosages expressed a significant improvement in GPx level indicating a dose dependent reversal of decreased GPx level due to lead toxicity.

Wine Grape Pomace (WGP) is rich in polyphenols, which are known to function as powerful antioxidants. Supplementation with WGP helps alleviate oxidative stress induced by restraint and enhances the activity of antioxidant enzymes in the testis (Zhao *et al*., 2017). The increased GPx levels observed with *Cucurbita pepo* L. seed extract may be attributed to its potent bioactive compounds, such as polyphenols, which contribute to its antioxidant properties.

viii)Testis Lipid Peroxidation (LPO)

Testis Lipid Peroxidation level of control and treatment groups upon *Cucurbita pepo* L. seed aqueous extract supplementation is represented in following Table XXIX.

Table XXIX

Testis LPO Control and Treatment Groups

Groups	Treatment	Testis LPO (nmol/mg)
CONTROL	Standard diet	2.835±0.214
LA	Lead acetate alone (30 mg/kg)	6.189±0.736***
PSE	*Cucurbita pepo* seed extract alone (1000 mg/kg)	1.153±0.424**
LA+PSE LD	Lead acetate (30 mg/kg) + *C. pepo* seed extract low dosage (100 mg/kg)	4.825±0.416**
LA+PSE HD	Lead acetate (30 mg/kg) + *C. pepo* seed extract high dosage (1000 mg/kg)	3.817±0.225*

*******p<0.001, **p<0.01, *p<0.05, ns-not significant***

Statistical comparison: Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett's comparison was performed. Treated groups were compared with control group.

The study demonstrated a significant increase in lipid peroxidation (p<0.001) in the lead acetate-treated group (LA) compared to the control group. Treatment with *Cucurbita pepo* L. seed aqueous extract alone resulted in a notable reduction in lipid peroxidation when compared to the control group. Additionally, co-treatment of lead acetate with *Cucurbita pepo* L. seed aqueous extract at both low and high doses led to a dose-dependent significant decrease in lipid peroxidation.

Aksu *et al.*, (2017) observed that pomegranate juice reduced lead levels in soft tissues and alleviated oxidative stress by lowering lipid peroxidation. Supplementation with pomegranate juice also enhanced the activity of antioxidant enzymes in lead-treated groups, showing a pronounced protective effect on the testis by virtue of their potent bioactive compounds. This supports the strong antioxidant activity of *Cucurbita pepo* L. seed extract in combating lead-induced oxidative stress.

4.5.5. Histopathology of reproductive organs in experimental male rats

Histopathology is the study of examining and interpreting the shapes, sizes, and structural patterns of cells and tissues within a specific clinical context. It is a scientific discipline that involves analyzing tissue images in relation to pathobiology to make an accurate diagnosis. In simpler terms, histopathology involves diagnosing and studying tissue diseases, primarily by examining tissues and cells under a microscope (Adyanthaya and Jose, 2013).

i) Histopathological examination of testis tissue harvested from control and treatment groups (Plate 7).

At the conclusion of the study, the testes from the sacrificed rats were removed to examine histological alterations if any. The histoarchitecture of the testis was inspected under a microscope in order to detect spermatozoa, Leydig cells, Sertoli cells, and germinal epithelium.

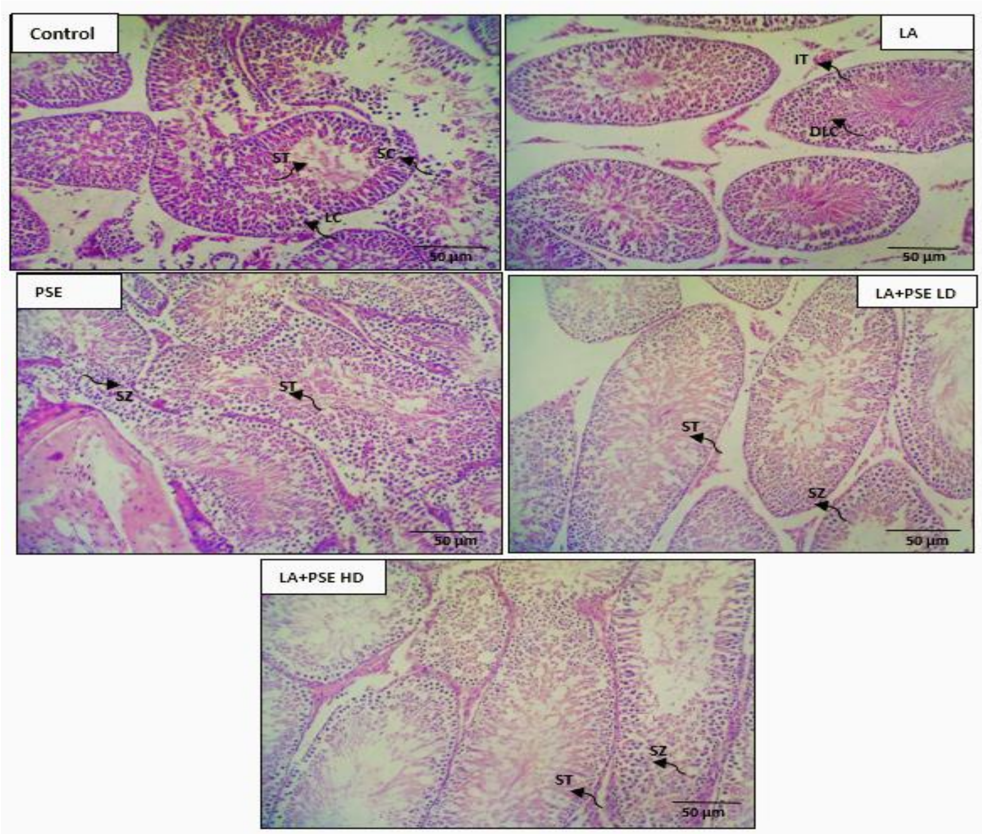

Plate 7: Histopathology of Testis

(H&E staining with magnification of 400x) Scale bar=50 µm

Control: Standard diet

LA: Lead Acetate alone

PSE: Pepo Seed Extract alone

LA+ PSE L.D: Lead acetate+ Pepo Seed Extract Low Dosage

LA+ PSE H.D: Lead acetate+ Pepo Seed Extract High Dosage

The testis photomicrograph of the control group revealed that the lumen of the seminiferous tubules (ST) contained an optimal number of spermatozoa. The germinal epithelium was seen in its typical size and form. In close proximity to the seminiferous tubule's basement membrane, the Sertoli cells (SC) were visible as a less dense mass. Leydig cells (LC) were seen to be normochromic between interstitial gaps. When compared to LA group, the *Cucurbita pepo* L. seed aqueous extract treated groups, PSE, LA+PSE LD and LA+PSE HD groups had densely packed lumen of seminiferous tubules with abundant spermatozoa (SZ). LA group showed a decrement in the number of Leydig cells (DLC) and spermatozoa with mild interstitial thickening (IT) indicating impairment in normal spermatogenesis. PSE group showed an increased spermatozoa and normal spermatogenesis with varying stages of maturation. Co-treatment with seed extract in both low and high dosages showed a recovery in the interstitial thickening and Leydig cells were found in normal number. This is indicative of the potential of the *Cucurbita pepo* L. seed extract in the recovery of the tissues.

ii) Histopathological examination of prostate tissue harvested from control and treatment groups (Plate 8)

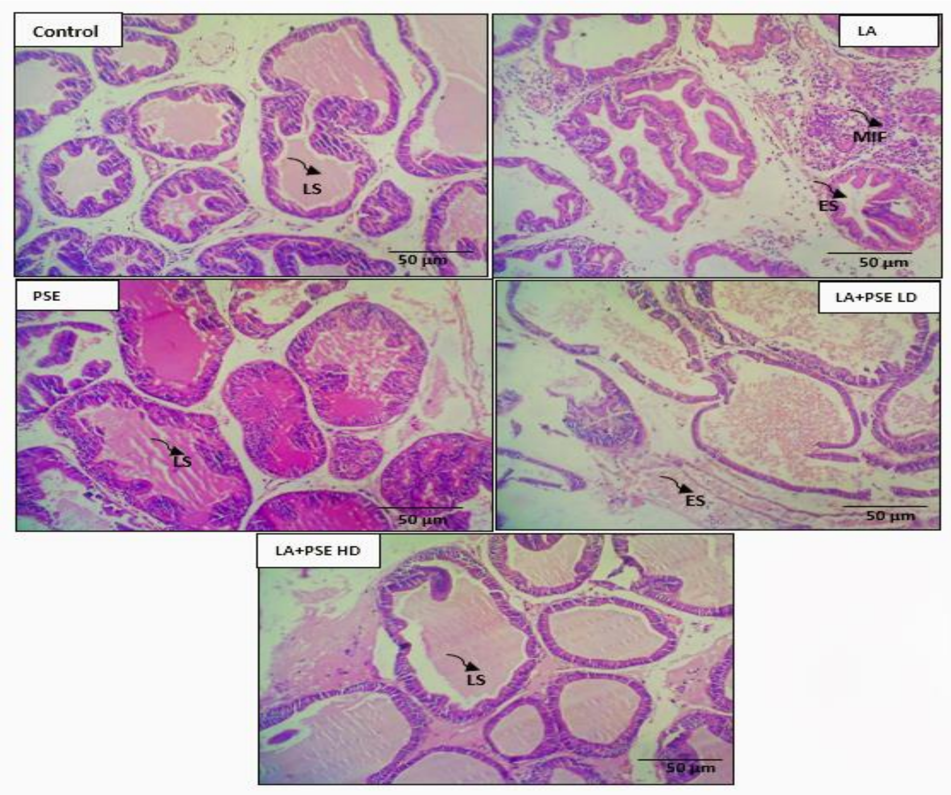

Plate 8: Histopathology of Prostate

(H&E staining with magnification of 400x) Scale bar=50 µm

Control: Standard diet

LA: Lead Acetate alone

PSE: Pepo Seed Extract alone

LA+ PSE L.D: Lead acetate+ Pepo Seed Extract Low Dosage

LA+ PSE H.D: Lead acetate+ Pepo Seed Extract High Dosage

Histopathology of prostate indicated that LA group showed edematous surrounding stroma (ES) and scattered mononuclear inflammatory infiltrates (MIF) whereas control and PSE showed normal architecture characterized by acini lined by simple columnar epithelium and basal cells luminal secretions (LS). LA+PSE LD showed edematous stroma but there was a reduction in the mononuclear inflammatory infiltrates. However, PSE HD exhibited stroma with normal luminal secretions indicating the effect of the extract in high dosage restoring the normal architecture of the prostate.

iii) Histopathological examination of caudal epididymis tissue harvested from control and treatment groups (Plate 9)

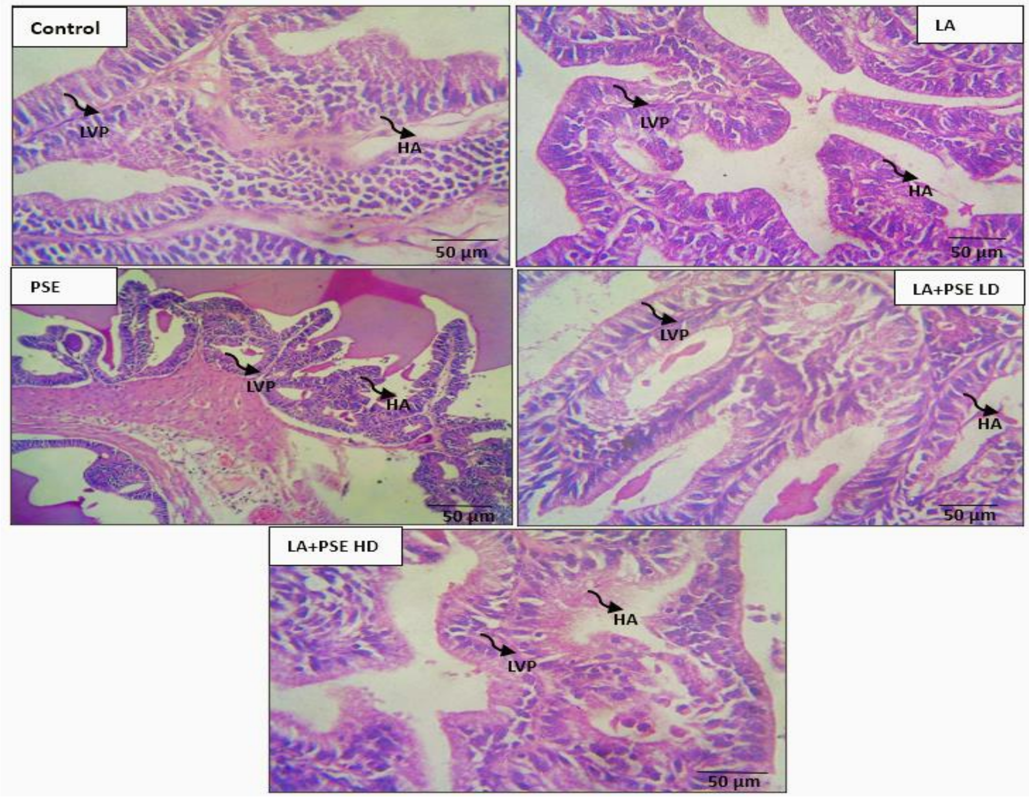

Plate 9: Histopathology of Caudal Epididymis

(H&E staining with magnification of 400x) Scale bar=50 µm

Control: Standard diet

LA: Lead Acetate alone

PSE: Pepo Seed Extract alone

LA+ PSE L.D: Lead acetate+ Pepo Seed Extract Low Dosage

LA+ PSE H.D: Lead acetate+ Pepo Seed Extract High Dosage

Histopathology of caudal epididymis indicated that all groups showed normal architecture characterized by hyperplastic acini (HA) lined by simple columnar epithelium and basal cells with luminal villi projections (LVP) filled with secretions. While the caudal epididymis may not show overt histopathological changes, sperm quality can still be significantly compromised by factors such as oxidative stress, hormonal imbalances, and functional disruption at the molecular level, even in the absence of structural damage.

iv) Histopathological examination of seminal vesicles tissue harvested from control and treatment groups (Plate 10)

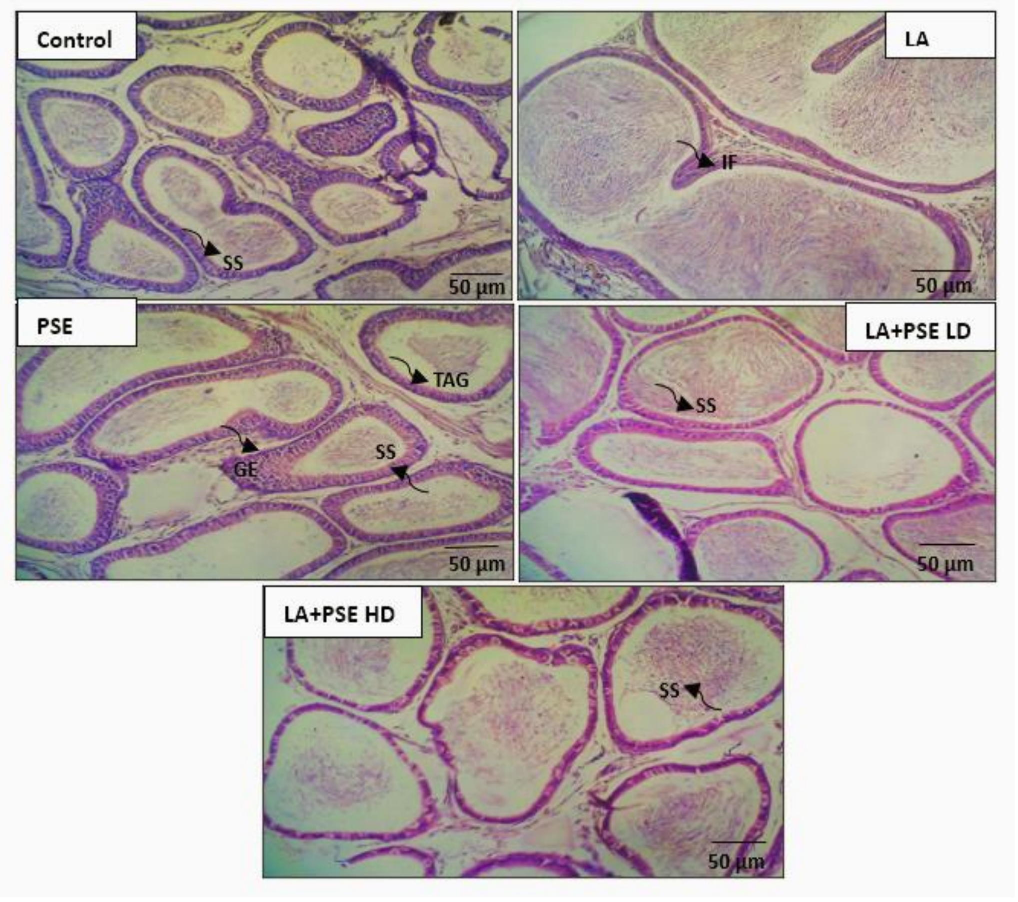

Plate 10: Histopathology of Seminal Vesicle

(H&E staining with magnification of 400x) Scale bar=50 µm

Control: Standard diet

LA: Lead Acetate alone

PSE: Pepo Seed Extract alone

LA+ PSE L.D: Lead acetate+ Pepo Seed Extract Low Dosage

LA+ PSE H.D: Lead acetate+ Pepo Seed Extract High Dosage

The efficient functioning of seminal vesicle is imperative for fertility. Histopathology of seminal vesicle revealed degenerative changes such as glandular alterations and inflammatory infiltrates (IF) in lead acetate alone treated group (LA). *Cucurbita pepo* L. seed aqueous extract alone (PSE) showed normal glandular epithelium (GE), tubule-alveolar glands (TAG), smooth muscle and supportive stroma (SS). Co-treatment with *Cucurbita pepo* L. seed aqueous extract showed signs of dose dependent restoration in the histoarchitecture as evident from normal glandular epithelium, tubule-alveolar glands, smooth muscle, supportive stroma in LA+PSE LD and LA+PSE HD groups.