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Adverse Effects of Propranolol on Testis and Heart Tissue of Albino Mice

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ABSTRACT

Propranolol is a non-selective, beta-adrenergic antagonist drug useful in various clinical disorders such as schizophrenia, anxiety, agitation, Autism Spectrum Disorder (ASD), hypertension, insomnia and other psychosis. **Objective:** To investigate the probable toxic effects of propranolol on the testis and heart of male mice. **Methods:** Twenty mature male mice were randomized and divided into four groups. The control group received distilled water, and the three experimental dose groups, D-I, D-II, and D-III, received 0.1 ml of propranolol using an insulin syringe with a butterfly needle in three different concentrations i.e. 0.15 µg/g, 0.30 µg/g, and 0.60 µg/g of their body weight, respectively, for a period of four weeks. After completion of the experiment, these mice were weighed, anaesthetized, and dissected. Their serum was collected by the intracardial puncture technique for the analysis of testosterone. Testes and heart were separated from the control and experimental groups for morphometric and histopathological studies. **Results:** The mice exposed to propranolol revealed a significant reduction in testosterone ($P \leq 0.01$) and body weight ($P \leq 0.01$). Histopathological findings also revealed laminar/tubular degeneration, vaccination, and an enlarged amyloid body in testicle tissues, while damaged striated strips and irregular arrangements of nuclei were observed in heart tissues. **Conclusions:** This investigation clearly reveals the adverse effects of propranolol on the testis and heart tissue of albino mice.

INTRODUCTION

The extent to which a substance harms living things is known as its toxicity. It could act on an organ such as the liver (hepatotoxicity) or on a cell (cytotoxicity) [1]. A poisonous substance can cause cancer, tremors, and euplastic effects in humans or developing organisms [2]. Propranolol is an anti-hypertensive drug that also possesses anti-psychotic properties and inhibits cardiac channels and neural voltage-gated channels, which is utilized to treat angina pectoris [3]. Furthermore, Propranolol helps to ease migraines. It also plays a role in stabilizing membranes or preventing arrhythmias [4]. It has been shown that propranolol blocks cardiac channels and skeletal muscles [5]. Propranolol hydrochloride, Dociton, β-propranolol, Euprovasin, Propranolol HCl, Inderal, Avlocardyl, and Herzbase are the some of the variants of chemical names for it [6]. Propranolol directly affects Leydig cells and also causes potent inhibition of sperm motility as well as testosterone deficit and inhibition of spermatogenesis. Its long-term exposure may impair testicular vasculature and Leydig cell function resulting in denaturation of seminiferous tubules and a consequent decline in fertility [7]. In one investigation, male mice exposed to Inderal were crossed with normal female mice, and the outcome showed that the male mice's seminiferous tubules showed toxic effects and decreased fertility, meaning that the effect was on the germ cells and Sertoli cells [8]. Additionally, propranolol inhibits cell division. As was previously mentioned, propranolol inhibits male spermatogenesis. It also inhibits the process by which spermatocytes divide into developing germ cells, a process that typically results in azoospermia or oligospermia [9]. Research also reveals that propranolol has inhibitory effects on sperm motility [10]. Adrenergic monoamines may be implicated in the modulation of sperm motility by

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both a calcium dependent and a cyclic nucleotide-dependent mechanism. These traits make it an excellent candidate for development as a potential novel spermicidal product [11]. Medicines such as propranolol directly affect Leydig cells; as a result, ejaculation may not occur or be highly suppressed due to ganglion blockage, additionally propranolol treatment has been associated with a decrease in ovulatory and reproductive function, which may have a link to its deleterious effect on spermatogenesis [12]. As a complex regulatory center for the human reproductive system, the hypothalamus is home to numerous neurotransmitters that are involved in the release of gonadotropic hormones. As a result, these neurotransmitters are highly susceptible to the effects of drugs that directly affect brain cells [13]. Beta blockers such as propranolol cause a marked lowering in heart rate and its contractility, which can lead to a decreased cardiac output when taken long term as a result of decreased ventricular hypertrophy [14]. The current study was aimed to substantiate the adverse effects of propranolol, which may be the inducing factor for gonadal and cardiac issues.

METHODS

Trials on animals were conducted according to local and worldwide procedures. The method followed in this study was the Wet op de dierproeven (article 9) of Dutch law (international) and similar laws regulated by the Bureau of Animal Experiment Licensing, of our University. The rearing and use of mice were carried out using the NIH Publication, 'Guide for the Care and Use of Laboratory Animals'(NRC, 2004) as detailed in our paper [15, 16, 17].

In Animal Rearing there were 20 albino mice, obtained from Veterinary Research Institute, Lahore and housed in stainless steel cages. Mice were raised in optimum conditions of 26 ± 2°C and 45–55% relative humidity with controlled light (12h light/dark cycle) as in our article. For Dose Administration Propranolol is available with trade name Inderal, which comes indifferent doses such as 10mg, 20mg, 30mg etc. While for experimental purpose, propranolol of 15µg, 30µg and 60µg were measured with digital balance, then mixed into 10ml distilled water to make three dose concentrations such as low, medium and high dose (0.15µg, 0.30µg and 0.60µg) respectively. Finally, three doses were prepared and given to the male mice, 0.1ml each in the experimental group with the help of insulin syringe attached with butterfly needle at its top. To check its toxicity, all mice were randomized and divided into four groups as; 1. Control group supplied with Nestlé's water, 2. Dose group-I (D-I), supplied with low dose of 0.1ml propranolol (15µg/g of B.W.), 3. Dose group-II (D-II), supplied with medium dose of 0.1ml of propranolol (30µg/g of B.W.) and 4. Dose group-III (D-III), given high dose of 0.1ml of propranolol (60µg/g of B.W.) respectively. For Biochemical Analysis on completion of 4 weeks, all mice were weighed, anesthetized and dissected. For hormonal analysis blood samples were collected by cardiac perfusion using 3ml syringe and spun for serum isolations and stored at -80°C.

For Hormonal Analysis, using Bio Vision kits, the serum testosterone levels in albino mice were determined. By reconstituting the testosterone standard with 1 mL of Standard Diluent, a standard curve was produced. Before use, all reagents were warmed to room temperature (20–25°C). Samples of mouse serum which were taken and frozen at -80°C, were then thawed at 4°C or room temperature the following day. To get rid of particulates, samples were centrifuged for five minutes at 10,000 rpm. Microplate wells were filled with 10 µL of the sample or standard and 100 µL of the assay diluent. After two hours of incubation, the plate was cleaned five times with Wash Buffer and treated with HRP Conjugate and detection antibody. Substrate Solution was added, and the plate was then incubated for 15 to 30 minutes. A microplate reader was then used to detect absorbance at 450 nm. The standard curve was used to determine the concentrations of testosterone. Regarding Morphological Observations, after dissections, Testis and heart were recovered from all mice for morphometric, histopathological studies, then fixed for 48 hours in Bouin's fixative. Finally, they were stored in 70% alcohol after 48 hours of fixation. In addition to Morphometric Observations, for morphometric and morphological analysis, the weight of testis and heart were measured with the help electric balance. Their diameter and lengths were also measured with the help of Vernier calipers. Afterwards the organs were taken to be micro photographed with the help of close-up lens fitted with Panasonic DMC-TZ15, Microscope and Camera, CZM6 of Japan for detailed measurements such as lumen and epithelial lining of seminiferous tubules. In Micrometry, twenty approximately circular, randomly selected seminiferous tubules which are much smaller than the whole testis, were traced from 400X photomicrographs in order to undertake their micrometric measurements. First, the seminiferous tubules' cross-sectional area (STA) was acquired by measuring the radius (r) of the tubule up to epithelium. This was done using photomicrographs taken by the microscope and camera and then analyzed via Image J software for assessing the dimensions of the tubules in mm2 [18]. The formula used to compute STA was ¼ πr2, where r is the tubule radius. Then, luminal radius (Lr) which is the radius excluding the epithelium and represented by the equation LA=¼ πLr2, was used to determine the area of the tubular lumen (LA) [19]. Subtracting LA from STA yielded the epithelial area (EA). The expression for the results was in square millimeters (mm2) (STA – LA = EA). Measuring Immotile Sperms viability testing is normally advised when determining whether non-motile sperm are dead or alive when sperm motility is lower than 5%–10%. Two techniques for assessing viability are the Hypo-Osmotic Swelling (HOS) test and dye exclusion tests. The Hypo-Osmotic Swelling (HOS) test was utilized to assess the membrane integrity of deceased sperm. A sample of

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dead sperm was mixed with 1.0 mL of a hypo-osmotic solution (7.35 g sodium citrate, 13.51 g fructose in 1 L distilled H20). The mixture was incubated for thirty to sixty minutes at 37°C. After incubation, 100–200 dead spermatozoa were inspected under a phase contrast microscope. The percentage of dead spermatozoa out of the non-motile sperms with no tail changes (no swelling, HOS-negative) was determined. Dead spermatozoa can be easily distinguished from living sperm through Trypan Blue staining which works by staining those cells that have compromised membranes thus indicating non-viability. Trypan Blue, as the name indicates, gives blue color to dead cells. Dead sperm with ruptured membranes received the Trypan blue dye and appeared blue, whereas healthy sperm with intact membranes rejected it [20]. In Smear Preparations for Sperm Count a midline incision was done, the testes and related epididymis were removed in saline solution, the curdy material used for the sperm count was removed, and a glass rod was used to gently crush it on clean glass. The chamber counting approach was used to examine the sperm morphologically. The total number of sperm-containing head and tail was counted once per four big 1 mm2 quadrates under a light microscope. Regarding Histopathology, organs were fixed in Bouin's fixative for 48 hours. Then these organs were kept in 70% alcohol. Afterwards, dehydration was done with 90% and 100% alcohol. Then organs were kept in cedar oil overnight then washed with Xylene. 50% wax and 50% Xylene were introduced in these tissues and then were kept in incubator overnight. Then 100 % wax was introduced into it and then incubated for 24 hours. Microtomy was done to cut the histological sections of 4-micron thickness. For staining purpose, Hematoxylin and Eosin stains were used to stain the slides, and then observed under the microscope at 400X following established protocol [21]. The Data was collected and referenced as in the form of tables and in text as mean ± standard error of mean and various parameters were compared using the one-way ANOVA Duncan test to compare them. Differences taken as significant when they were at $p \leq 0.05$.

RESULTS

Regarding Morphometric Studies, the organs from all the groups; control and experimental, were subjected to morphometric analysis. In control group, the average weight of testis stood at 0.015 ± 0.07, while in Group I, the average weight of testis was 0.019 ± 0.22which had significantly increased (P<0.05). The average weight of testis in Group II was 0.024 ± 0.23 which had significantly increased (P<0.01) as compared to control group. Whereas, the average weight of testis of Group III was 0.13 ± 0.09, which was significantly increased (P<0.001) as compared to control group (Table 1). The average testis length and width in Control Group was (6.02 ± 0.98 and 3.10 ± 0.24) which was in normal range. While in Group I, the average testis length and width was (4.13 ± 0.19 and 3.87 ± 0.78) which was significantly increased (P<0.05) as compared to Control group. The average length and width of testis in Group II was (6.53 ± 0.23 and 3.96 ± 0.34) which was significantly increased (P<0.01) The average length and width of testis in Group III was (7.12 ± 0.26 and 3.98 ± 0.35) which had increased more significantly (P<0.001) as compared to control group (Table 1). Gonado-Somatic Index (GSI), the Gonado-Somatic Index is used to assess the reproductive information of animals. It is a ratio of the weight of gonads to the total body weight and helps to assess the changes in gonad size as a result of chemical administration. Since it is a ratio it does not have units. It was measured by weighing the whole body and then the testis alone on an electronic weighing machine. In control group the average Gonado-Somatic Index stood at 0.07 ± 0.12 while in Group I, the average Gonado-Somatic Index was 0.08 ± 0.14 which increased significantly (P<0.05). The average Gonado-Somatic Index in Group II was 0.097 ± 0.21, which was significantly increased (P<0.01) as compared to control group. Whereas, the average Gonado-Somatic Index in Group III was 0.099 ± 0.08 which was significantly increased (P<0.001) as compared to control group as well (Table 1). In control group the average weight of heart stood at 0.036 ± 0.04, while in Group I. The average weight of heart was 0.036 ± 0.04 which increased significantly (P<0.05). The average weight of heart in Group II was 0.049 ± 0.18, which was significantly increased (P<0.05) as compared to control group. Whereas, the average weight of hearts of Group III was 0.049 ± 0.18, which was significantly increased (P<0.001) as compared to control group (Table 1). The average heart length and width in control group was 3.02 ± 0.08 and 1.87 ± 0.29 which was in the normal range. While in Group I, the average heart length and width was 4.13 ± 0.19 and 3.60 ± 0.35 which was significantly increased (P<0.05) as compared to control group. The average length and width of hearts in Group II were 5.02 ± 0.33 and 4.21 ± 0.67 which were significantly increased (P<0.01) as compared to control. The average length and width of hearts in Group III were (6.08 ± 0.44 and 4.41 ± 0.45) which were significantly increased (P<0.001) as compared to control group (P<0.001) (Table 1).

Table 1: Morphometric Investigations of Propranolol Induced Toxicity in Testis and Heart of Male Albino Mice

Variables	Control	Group-I	Group-II	Group-III
Testes Weight (mg ± S.E.)	0.015 ± 0.07	0.019 ± 0.22*	0.024 ± 0.23*	0.13 ± 0.09***
Testes Length (cm ± S.E.)	6.02 ± 0.98	6.43 ± 0.87	6.53 ± 0.23*	7.12 ± 0.26**
Testes Width (cm ± S.E.)	3.10 ± 0.24	3.87 ± 0.78*	3.96 ± 0.34**	3.98 ± 0.35**
Gonado-Somatic Index	0.07 ± 0.12	0.08 ± 0.14	0.097 ± 0.21**	0.099 ± 0.08**
Heart Weight (mg ± S.E.)	0.036 ± 0.04	0.046 ± 0.19*	0.049 ± 0.18*	0.092 ± 0.22**

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Heart Length (cm ± S.E.)	3.02 ± 0.08	4.13 ± 0.19*	5.02 ± 0.33**	6.08 ± 0.44***
Heart Width (cm ± S.E.)	1.87 ± .29	3.60 ± 0.35**	4.21 ± 0.67***	4.41 ± 0.45***

For serum testosterone level and apoptotic cells per seminiferous tubule, the average serum testosterone level in control group was 1.98 ± 0.06 ng, which was in normal range. While in Group I was decreased to 1.1 ± 0.09 ng. In Group II, was 1.62 ± 0.07 ng which was decreased as compared to control group? Whereas due to high dose in Group III, the level of serum testosterone was substantially decreased to 1.04 ± 0.28 ng revealing toxic effects of propranolol on male reproductive system of male albino mice (Figure 1). Apoptotic bodies were identified from the already stained seminiferous tubules and counted. They were observed under microscope, with apoptotic cells possessing symptoms for identification such as condensed nuclei and fragmented cells. They were measured as; number of apoptotic bodies per 20 seminiferous tubules. The average apoptotic cells per seminiferous tubule in control group was 0.2 ± 0.12. In Group I, it was increased to 0.8 ± 0.18. In Group II, it was 1.98 ± 0.24 which was increased as compared to control group. Whereas due to high dose in Group III, the average apoptotic cells were 3.05 ± 0.8 which was more significant than in control group revealing toxic effects of propranolol on male reproductive system of male albino mice (Figure 1).

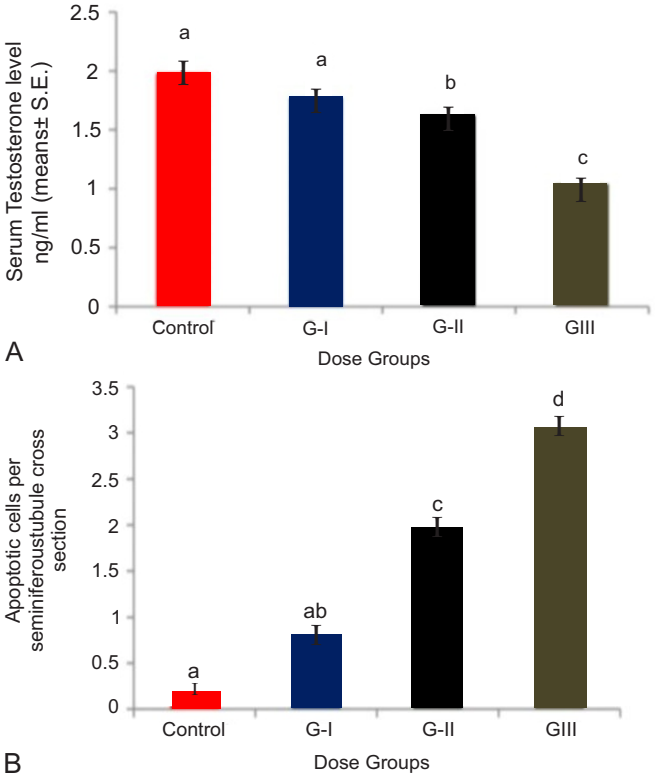

Figure 1: Plotted graphs A and B; shows the significant reduction of Serum testosterone levels and significant increase of apoptotic bodies in seminiferous tubules after propranolol exposure for a period of 28 days. Significant difference in all groups at the significance value ($p < 0.05$) represented by the addition of various alphabets as superscripts.

In Epithelial and Lumen Diameter of Seminiferous Tubules, the average epithelial diameter of seminiferous tubules in control group was 78.98 ± 0.17 µg. In Group I was decreased to 77.17 ± 0.19 µg. In Group II, it was 80.29 ± 0.41µg which was increased as compared to control group. Whereas due to high dose in Group III, the average epithelial diameter was 81.15 ± 0.81µg, which was more significant than control group revealing toxic effects of propranolol on male reproductive system of male albino mice (Figure 2). The lumen diameter of seminiferous tubules in control group was 80 ± 0.19µg. In Group I, it was decreased to78.01 ± 0.17 µg. In Group II, it was 82 ± 0.18 µg which was increased as compared to control group. Whereas due to high dose in Group III, the average lumen diameter was 84.28 ± 0.19 µg which was more significant than control group revealing toxic effects of propranolol on male reproductive system of male albino mice (Figure 2).

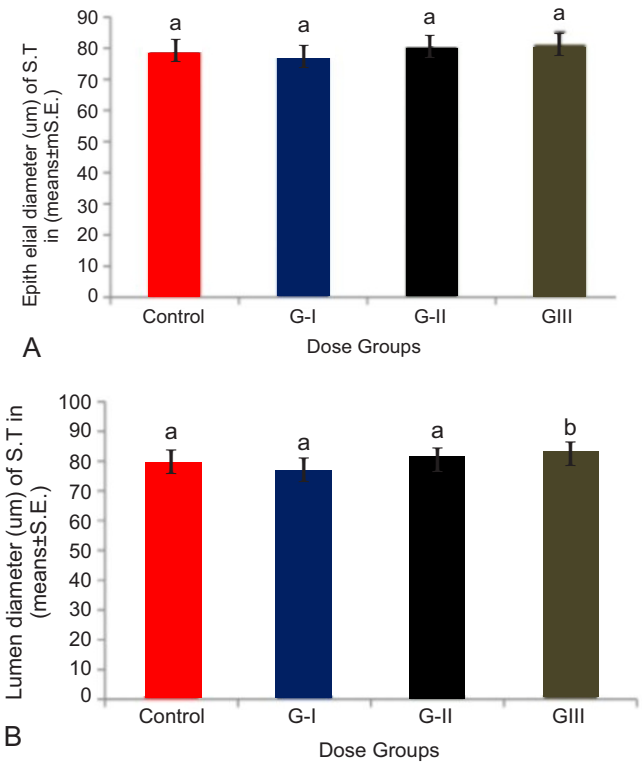

Figure 2: Plotted graphs A and B, showing the significant increase in epithelial and lumen diameter of seminiferous tubules after propranolol exposure for a period of 28 days. Significant difference in all groups at the significance value ($p < 0.05$) represented by the addition of various alphabets as superscripts

The Percentage of Motile and Immotile Sperms in control group was 70 ± 0.19. In Group I, it was decreased to 69 ± 0.63, in Group II was 71 ± 0.41, which was increased as compared to control group. Whereas due to high dose in Group III, the

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average lumen diameter was72 ± 0.21indicating that propranolol has no significant toxic effect on sperm motility (Figure 3). Therefore, Control and high dose group i.e., Group III showed significant difference (P<0.05). Immotile Sperm Percentage in control group was 20%, while in Group I was decreased to 18% and in Group II they dropped to 17%. Whereas in Group III, sperm percentage was 18% which indicated that there is no significant effect of propranolol on sperm motility according to this study (Figure 3).

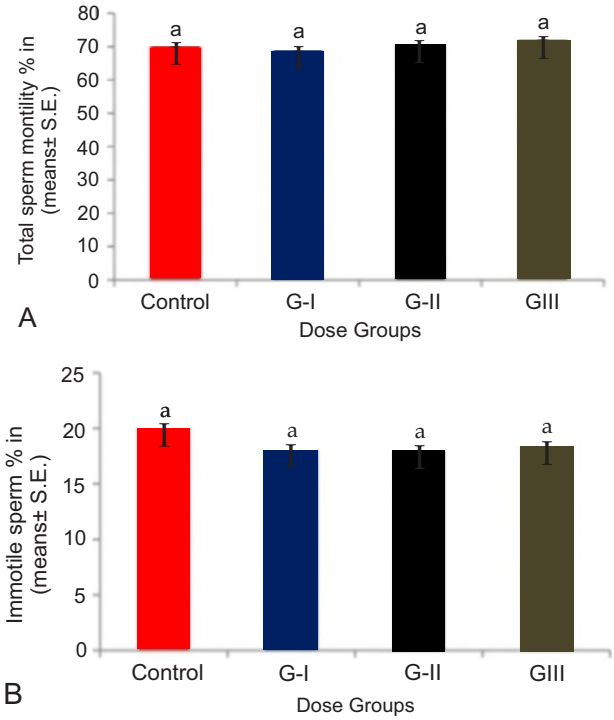

Figure 3: Plotted graphs A and B, showing the non-significant decrease of sperm motility and immotility in seminiferous tubules after propranolol exposure for a period of 28 days. (Changes occurred but were not significant, represented by the addition of various alphabets as superscripts).

Histopathological findings of testes (Figure 4) revealed normal structures in control group as normal seminiferous tubules were seen as well as normal spermatids. Contrarily, in Group-I low dose of propranolol damaged testes tissue as vacuolization and degeneration in tubules was observed. In Group II, propranolol dose increased the damaging effect on testis as reduced lamina, tubular degeneration and vacuolization were observed. Finally, in Group III, propranolol's high dose augmented the damaging effect as poor tubular formation and enlarged amyloid plaques were observed indicating that propranolol had damaging effects on gonads.

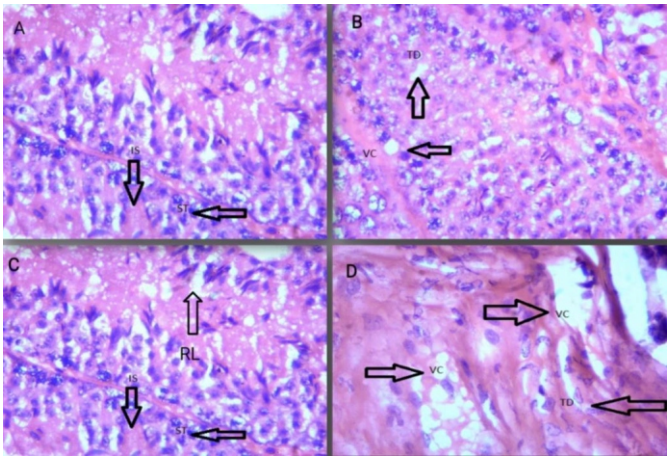

Figure 4: Histopathological sections of testis at 400X of A (Control group), B (Group-I), C (Group-II) and D for (Group-III). ST=Seminiferous tubules, IS=Interstitial spaces. VC=vacuolization, RL=Reduced lamina, TD=Tubular degeneration and AB=Amyloid body.

Histopathological findings of heart tissue (Figure 5) revealed normal striated strips in Control group. While in Group-I, low dose of propranolol damaged heart tissue as damaged strips and irregular nuclei were observed. In Group-II, medium dose of propranolol affected tissue necrosis and vacuolization was more than Group-I. In Group-III, water accumulated between the damaged strips. So, more vacuolization and damaged strips were found in Group-III as propranolol usage revealed toxic effects on heart tissues.

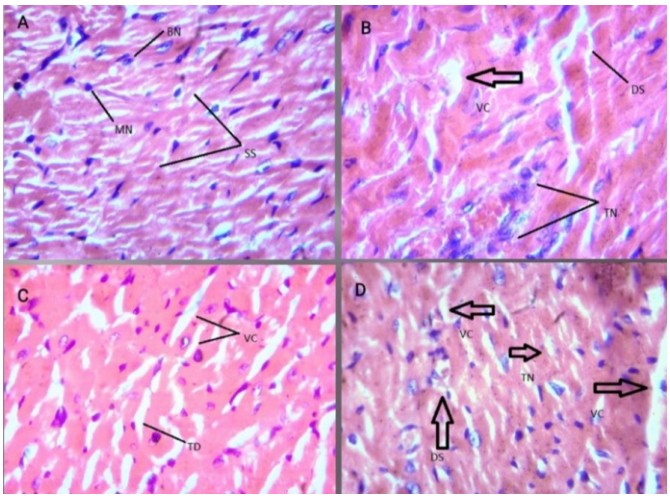

Figure 5: Histopathological sections of heart at 400X of A (Control group), B (Group-I), C (Group-II) and D for (Group-III). SS=Striated strips, MN=Mononucleate, BN=Binucleate, TN=tissue necrosis, DC=damaged strips and VC=Vacuolization.

DISCUSSION

Propranolol is an antihypertensive drug also having antipsychotic properties; it also blocks the neuronal voltage-gated channels and the cardiac channel. It is used for the treatment of angina pectoris, anxiety, and

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hypertension. Due to its wide use, there was a need to assess its toxic effect on various organs of the body. In our study effect of propranolol on the somatic and germ cells of albino mice was investigated. In a study on drug effect on mice tissues, it was found that while chromosomal abnormalities were induced in the germ cells, the frequency of nuclei in the cardiocytes was significantly elevated [22]. In our study, mice in experimental groups received a daily dose of 0.15µg/B.W., 0.30µg/B.W., and 0.60µg/B.W. Throughout the four weeks, administration of propranolol resulted in deleterious effects on various body parts, such as the testis, and heart size increased. In contrast to previous studies; propranolol treatment has been demonstrated to limit hypertrophy and an increase in heart weight, which can have detrimental consequences on cardiac function and general health [23] aligned with our findings that the body weights also increased markedly throughout the four weeks. Biochemical findings revealed that a high dose of propranolol decreased the level of testosterone, these results are justified by another study that taking propranolol may cause sperm motility to decrease [24]. Whereas histopathological findings showed destruction in testes and heart tissues, as vacuolization, tubular degeneration and amyloid bodies were found in testicular tissue, and damaged striated strips and irregular arrangements of nuclei were found in heart tissue. Likewise, in other studies in which testicular tissues of the propranolol-using groups showed vacuoles and necrosis in the germinal epithelium, as well as blood vessel congestion in the interstitial space [25]. The findings correlated with previous evidence in which propranolol use over an extended period of time has been connected to cardiac hypertrophy, which raises the risk of heart failure as well as further heart issues mostly the risk of weakened musculature [26].

CONCLUSIONS

The present investigations reveal that high dose of propranolol does indeed have harmful effects on testes and heart tissue. High doses resulted in the deterioration of sperm health and fertility by affecting the seminiferous tubules while also influencing cardiac health by limiting ventricular hypertrophy and increasing lumen diameter which is characteristic of beta-adrenergic drugs. It also causes vacuolization and necrosis in cardiac tissue. Hence should be avoided for extended use in animals with possible scope for further research in human subjects.

Authors Contribution

Conceptualization: A
Methodology: ML
Formal analysis: AA
Writing, review and editing: JS, AA, AUMF, HF, FA, MRF

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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