A biochemical approach to evaluate the toxic effect of Pyriproxyfen on reproductive system of adult male Sprague Dawley rats

By

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A dissertation submitted in the partial fulfillment of the

Requirements for the Degree of Master of Philosophy

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“In the Name of Allah, the most Beneficent, the most Merciful”

CERTIFICATE

This dissertation "A biochemical approach to evaluate the toxic effects of Pyriproxyfen on reproductive system of adult male Sprague Dawley rats.." submitted by **Ms. Tahira Iram** is accepted in its present form by the Department of Zoology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirement for the degree of Master of Philosophy in Reproductive Physiology.

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DECLARATION

I hereby declare that the work presented in the following thesis is my own effort and the material contained in this thesis is my original work. I have not previously presented this work elsewhere for any other degree "**A biochemical approach to evaluate the toxic effect of Pyriproxyfen on reproductive system of adult male Sprague Dawley rats**."

Tahira Iram

DEDICATION

Dedicated to My Parents who have given me the

Opportunity to Study from the best institutions and

have Supported me throughout my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Dl	Deciliter
LDL	Low-density-lipoprotein
HDL	High-density-lipoprotein
ELISA	Enzyme-linked immunoassay
ECM	Extracellular Matrix
HRP	Horse-radish Peroxide
TMB	Tetramethylbenzidine
DSP	Daily sperm production
FFA	Free fatty acids

LPL	Lipoprotein lipase
ATP	Adenosine triphosphate
GK	Glycerol kinase
G-3-P	Glycerol-3- phosphate
ADP	Adenosine diphosphate
GPO	Glycero-phosphate oxidase
POD	Peroxidase
H202	Hydrogen peroxide
RB	Reagent blank
CAL	Calibrator

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I owe my gratitude to the One who is the Most Beneficent, Altruistic and Merciful, **Almighty Allah**, Who puts the sun's seal on the tablets of the flowing waters and throws clouds to the skies, Who distills the waters of the clouds over the seas to conceive the pearl in the womb of the oyster, Who creates fire in every stone, color in the fire, radiance in the color, Who gives voices to the dust, word to the voices, and life to the world, Who created us as a Muslim and blessed us with knowledge to differentiate between right and wrong. All prays to Him as He blessed us with the **Holy Prophet, Hazrat Muhammad** (SAW) for whom the whole universe is created and who enabled us to worship only one God. He (SAW) brought us out of darkness and enlightened the way of heaven

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Tahira Iram

Abstract

Pyriproxyfen is a fenoxycarb derivative a juvenile hormone analog. Pyriproxyfen causes unpropitious effects on vital organ of rodents such as kidney, pancreases and liver. In previous studies, it is noted that pyriproxyfen causes endocrine and reproductive toxicity. The objective of this study is to calculate harmful consequences induced by pyriproxyfen in male Sprague Dawley rats. The experiment was conducted for 28 days. Rats were distributed into four groups (n=5). Group-I considered as control, received normal saline. Group –II treated with pyriproxyfen 64mg/kg, Group-III was given 124mg/kg, and Group-IV was given 186mg/kg orally. All the animals were weighed and sacrificed by decapitation on day 29. Plasma was extracted from blood samples and kept at -20°C for biochemical examination. Pyriproxyfen non-significantly decrease the body and accessory organ weights. While the weight of testis decreases significantly. Pyriproxyfen administration induce a notable ($p<0.001$) decrease in DSP. in treated groups remarkable reduction in blood glucose concentration ($p<0.001$) was detected. The results showed that biochemical markers serum total protein content, cholesterol, LDH, Triglycerides increased significantly. Pyriproxyfen-treated groups displayed a significantly lower testosterone concentration as compared to control group. Plasma cortisol level increased significantly in high dose treated groups. The study concluded that pyriproxyfen decreases body and accessory organ weight, increases oxidative stress and reproductive dysfunctions in testis

Introduction

Endocrine disruptors are exogenous chemicals that hinder the hormone activity (Zoeller *et al*., 2012). According to the EPA of the United States, endocrine disruptors are the chemicals, introduced into the body, have the potential to alter endocrine and homeostatic systems by interfering with the receptor binding or elimination of endogenous hormones production, transport and metabolism (Mnif *et al*., 2011; Kiyama *et al*., 2015). The European Commission proposed that the standard to designate EDC should require evidence of three actions: (1) lethal and pathologic endocrine mediated function; (2) Endocrine activity (3) cause-effect relationship between substance and endocrine action (Slama *et al*., 2016).

European Food Safety Authority (EFSA) affirmed that most EDCs are synthetic molecules, interact with hormone receptors and control gene expression. DNA methylation, acetylation, and histone modifications are examples of epigenetic modifications that are involved in endocrine disruption pathways(Zama *et al*., 2010).EDCs can be categorized on the bases of their origin as follows: Industrial (including dioxins, alkyl phenols, and polychlorinated biphenyls) agricultural (fungicides, pesticides, phytoestrogens, insecticides, herbicides), residential (polybrominated biphenyls, bisphenol A, phthalates) pharmaceutical (parabens) and industrial (dioxins, alkyl phenols, and polychlorinated biphenyls).

Some of the heavy metals such as lead, cadmium, arsenic and mercury also considered as EDCs (Lubrano *et al*., 2013). They may contaminate the human body through many paths. The most common pathways of exposure include food intake, inhalation, and direct contact. Newborns get contaminated with EDC by breastfeeding and contact with baby products (Polyzos *et al*., 2011; Gore *et al*., 2015; Kabir *et al*., 2015; Balaguer *et al*., 2017). Being highly lipophilic nature, EDCs, accumulates in the adipose tissue—and generally has a long half-life. These two characteristics explain why EDCs can build up for years in any animal's adipose tissue, making co-contamination quite common. Many EDCs have complex dose- effect interactions that might result in divergent effects at various dosages relationships. Due to varied ways that each EDC bind to hormone receptors, hey each produces dose-dependent responses that are non-traditional (Barouki *et al*., 2017).

Mechanism of endocrine disruption

The actions of EDCs are mediated through direct contact with nuclear hormone receptors, or by binding and activating numerous hormone receptors (androgen receptor) and may mimic the natural activities of hormones (agonist action) (Heindel *et al*., 2015; Mnif *et al*., 2011; Monneret, 2017). They may induce the reduction of endogenic hormone concentrations by impeding the formation; transport, breakdown, and removal of hormones (Mnif *et al*., 2011; Thomas Zoeller *et al.,* 2012). EDCs impair the body's ability to effectively regulate the endocrine system, resulting inthe induction of developmental, reproductive, and neurological consequences. The association of EDCs with other EDCs enhances the harmful effects of EDCs (Nohynek *et al*., 2013). EDCs regulate all the major body systems affecting the endocrine axis, like HPA, HPG and HPT axis (Thomas *et al*., 2012).

The 1960s Green Revolution dramatically raised the agricultural production. Thousands of perilous compounds formulated to manage the agricultural pests and ensure high production and quality (Popp *et al*., 2013). They might have negative effects on non- target organism and the environment (Tiryaki and Temur, 2010; Pisa *et al*., 2015). The negative effects of extensive usage of these agricultural products on environment and human health, firstly reported in Rachel Carson's book ―Silent spring published in 1962 (Carson, 1962). Pesticides are vital endocrine disruptors (Vinggaard *et al*., 2000). Of which, 46% are insecticides, 31% fungicides and 21% herbicides. Even though many chemicals have been outlawed for decades, some of them can still be present in the surroundings (DDT).

Tens of thousands of formulations pesticides with more than 800 active chemicals are available globally (PAN-UK., 2009), each year 2 million tons of pesticides are used, resulting in 250,000 fatalities worldwide (Uggini *et al*., 2010; Yang CC *et al*., 2007). Pesticidesexposure cans happen via number of pathways such as dietary exposure to pesticide, residues from residential products and agricultural drift. Living near the facilities where these hazardous chemicals are manufactured, used and disposed of increased the contact to pesticides through inhalation and interaction with air, water and soil (Mantovani *et al*., 2008; Peakall, 2000). Some studies have revealed a link between pesticides exposure and diseases like allergies, neurological problems cancer and reproductive disorders. Since 2000 a lot of epidemiological studies clinched that pesticides effects spermatogenesis, lower the semen

quality and waned male fertility. Number of meta-analyses and case-control studies meta- analyses done to assess the risk of prostate cancer. Professional exposure increased the chances of prostate cancer between 10 and 40% (Parron *et al* 2010).

Classification of insecticides:

Insecticides are categorized into four classes based on their mechanism of action: (1) acetylcholine mimics (neonicotinoids) (Kathrina and Antonio 2004), nerve impulse targets which involve cholinesterase inhibitors (organophosphates and carbamates) (Lotti *et al*., 2000); Lotti *et al*., 2001), followed by sodium channel activators (DTT and pyrethroids) (Narahashi *et al*., 2000; Soderlund *et al*., 2012), chloride channel blockers (avermectins) (Huang and Casida 1997), Ca channel activators, Na channel inhibitors (piperidines and carbazones) (von Stein *et al*. 2013), and (diamides) (Lahm *et al*., 2009). (2) Mitochondrial agents (Isman *et al*., 2006), glycolysis inhibitors (arsenicals) (Bencko and Yan Li Foong 2017), energy production objectives, such as alkylating agents (methyl bromide) (De Souza *et al*., 2013; Bulathsinghala *et al.,* 2014). (3) Interfering growth regulators; (Mian and Mulla, 1982; Boudjelida *et al*., 2005), chitin synthesis inhibitors (Abo-Elghar *et al*., 2004; Gangishetti *et al*., 2008). (4) Miscellaneous (Glare and O'Callagham, 2000;Vachon *et al*., 2012).

Classification of pesticides

According to their intended target and mechanisms of action, pesticides are classified as either juvenile hormones (mimics the effects of insect hormones) or chitin synthesis inhibitors (Gangola S *et al*., 2015). Pesticides are divided into four major groups based on their chemical composition: organochlorine, organic phosphorous, carbamate, pyrethrin, and pyrethroid. Biopesticide is another class of pesticides; is subdivided into three main categories: plant-incorporated protectants, biochemical pesticides, microbial pesticides. Biopesticide often referred to as bio rational pesticides that have biological origin such viruses, bacteria, fungus or from biochemical like pheromones or insect growth regulators (IGRs).

Insect growth regulators are chemical compounds (Schneiderman invented the term "IGRs" in 1972) that inhibits the life cycle of insects, pests and induce changes in

development and growth. IGRs include lufenuron, fenoxycarb, methoprene, buprofezin, diflubenzuron, buprofezin, kinoprene, and Pyriproxyfen (Tunaz H *et al*., 2004).

Pyriproxyfen

In 1990s the Sumitomo Chemical Company first registered the Pyriproxyfen 2-{1-methyl-2-(4-phenoxyphenoxy) ethoxy) trademark (Sullivan and Goh, 2008).

Structure

It is a fenoxycarb derivative with achiral Centre and aliphatic chain that has had a portion of the chain replaced by pyridyl oxyethylene.

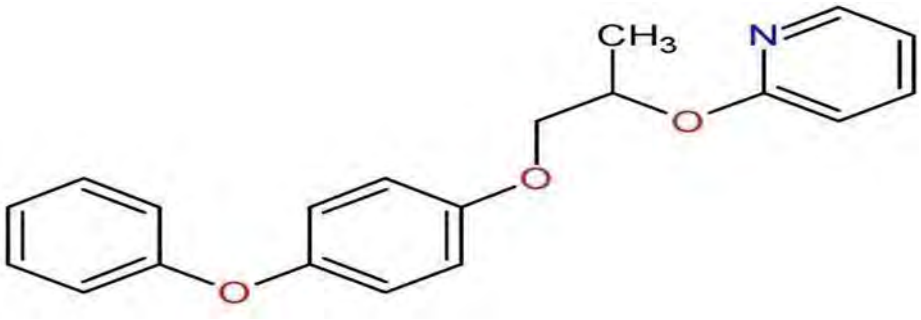


Fig: 1 Chemical structure of pyriproxyfen

Properties of Pyriproxyfen

Molecular and chemical formula of pyriproxyfen is 321.5 g/mol, C20H29NO3 respectively. It has a 5.3-inch diameter and a length when fully stretched of about 18.4 inches with melting point of 45 and 47 °C and 1.0 107 mm/Hg at 20 °C vapor pressure. It has a PHof 6 and 0.367 mg/l water solubility (Sullivan, J *et al*., 2000. In an aqueous buffer at 25 °C it is resilient against hydrolysis at 5, 7, and 9 pH in the dark but susceptible to photolysis (FAO 1999; Sullivan and Goh 2008). In aerobic and anaerobic water has a half-life of 23.1and 288.9 days respectively (Sullivan, 2000; Sullivan and Goh, 2008).

Uses and limitations of pyriproxyfen

It is frequently used for residential pest control and interior sanitation (Saltzmann *et al*. 2006).This juvenoid has been approved for use in agriculture and horticulture to controlthe red imported fire ant (Solenopsis Invicta) (Hwang 2009), silver leaf whitefly (Bemisia tabaci) and mulberry scale (Pseudaulacaspis pentagonal) (Isayama and Tsuda 2008). Numerous crops including coffee, pineapple, banana, papaya, avocado, almond, peanuts, orange, cotton, and apple have been treated with pyriproxyfen (Sullivan and Goh, 2008). Pyriproxyfen was to directly kill mosquitoes in surface water during the Zika virus outbreak. Pregnant women were suspected of microcephaly in babies in Brazil as a result of direct contact with pyriproxyfen containing water (Evans *et al*., 2016; Vazquez, 2016).

Eastern Spain Júcar River was found to contain 99.59 ng/L of pyriproxyfen (Belenguer *et al*., 2014). The WHO Pesticides Evaluation Scheme states that pyriproxyfen concentration in drinkable water should not be higher than 0.01 mg/L (Organization, 2006, 2018). A limit of 100 mg per 1 kg of body weight each day set by the World Health Organization (Truong *et al.*, 2016). At the Joint FAO/WHO Meeting on Pesticide Residues pyriproxyfen was not declared as genotoxic or carcinogenic compound (Invest and Lucas, 2008)

Metabolism of Pyriproxyfen

There are numerous ways in which pesticides might metabolise in the environment and produce various types of substances (Fenner *et al*., 2013); Bonmatin *et al*., 2015; Pisa *et al*., 2015). The environmental behavior and harmful effects of chiral pesticides enantiomers have garnered a lot of interest (Petrie *et al.*, 2015)

Intended metabolic pathways of pyriproxyfen have been seen in zebra fish (Kodaka *et al*., 2010; Liu *et al*., 2019b). It could break down via the CYP450 monooxygenase to PYR, 4′–OH–PYR and 5″–OH–PYR. 4′–OH–PYR was converted into DPH-PYR, PYPA, 4′–OH– POPA, POP. CYP450 isozyme may catalyze the breakdown of ether linkage (Ohe *et al*., 1994; Sträuber *et al*., 2003). PYPA further oxidized to PYPAC and finally to 2- hydroxypyridine. All these metabolites can be generated in animals and plant matrix.

Research revealed that hydroxylation metabolism of Pyriproxyfen was catalyzed by CYP6Z2, CYP2C, cytochrome P450 monooxygenase in rats, houseflies and zebra fish respectively (Yoshino *et al*., 1996; Zhang *et al*., 1998; Jones *et al*., 2008; Yunta *et al*., 2016).

Metabolites of Pyriproxyfen

In rats 4′ and 5″–OH–PYR is its metabolite (Matsunaga *et al.,* 1995; Yoshino *et al.,* 1995). One of the metabolites is 4′–OH–POP, structurally categorized as bisphenol, is more dangerous to earthworm than parent compound (Liu *et al*., 2019). 5″–OH–PYR is more toxic to rat hepatocyte (Liu *al*., 2019).

Toxicity

Pyriproxyfen is easy to store in fat leading to toxicity risk in animals. Pyriproxyfen revealed potential risks in different organisms like bees adverse behavior (Fourrier *et al*., 2015), mice reproduction toxicity (Shahid *et al*., 2019), developmental harms in Hippodamia convergens (Iftikhar et al., 2020), endocrine disturbance in land crab and Odontophrynus americanus (Linton *et al*., 2009; Lajmanovich *et al*., 2019). The ability of young Xiphophorusmaculatus to feed and swim would be reduced by suitable concentration of pyriproxyfen (Caixeta *et al*., 2016). Pyriproxyfen disturbs all the reproductive stages in insects (Barbosa *et al*., 2018; Maharajan *et al*., 2018; Meng *et al*., 2018). It causes death initial pupal stage (Lucas, 2008; WHO Pesticide Evaluation Scheme (WHOPES) & Hustedt *et al.,* 2017). In Daphnia assays for acute and chronic toxicity tests were created to examine inhibition of molting and feeding, mortality and fecundity (Trayler and Davis, 1996; Vieira-Santos *et al*., 2017). Mesocyclops pehpeiensis and Megacyclops Viridis longevity, fertility, mortality, and abilities were assessed (Wang *et al*. 2005b). There were no variations between the developmental stages. In M. Mesocyclops pehpeiensis many adults emerged, no difference observed between treated and control groups in terms of the number of egg-bearing events, predatoty behavior and sex ratio of each brood. During egg hatching stage high mortality, no impact on sex ratio, short development time, extended life span and predatory behavior and was examined. It enhanced the larval mortality and triggered behavioral changes in the Aedes aegypti population Ultrastructural examination reveal that pyriproxyfen caused morphological damage of midgut cells of Aedes aegypti larvae including Vacuolization, destruction to brush

border, lipid droplets rich basal cytoplasm, and malformed mitochondria (Hazarika *et al*., 2018; Costa *et al*., 2014; Vasantha-Srinivasan *et al*., 2018). Amphibians' populace is declining worldwide. Besides being sensitive to environmental toxins and low water pesticide pollution levels human actions put at risk this phylum of vertebrates, disrupting enzymes and hormones, (Bishop *et al*., 2012 Chanson *et al*., 2008). Pyriproxyfen being cardio toxic decreases the heartbeat in the O.americanus. The generation of reactive oxygen species (ROS) rise and may induce secondary toxic effects at cardiac and tissue levels (Güleç *et al*., 2006). The pyriproxyfen-treated exhibit neurotoxicity effects like low average speed and global activity. (Peltzer *et al*., 2013: Helbing *et al*., 2006). 4′OH-PPF interrupts thyroid signaling and behavior in Xenopus laevis (Spirhanzlova *et al*. (2018) According to the studies, this metabolite altered neuronal development, behavior, thyroid signaling as well as increased Musashi-1 expression (Spirhanzlova *et al*., 2018). Daily decreases in body weight, normalized hind limb length snout-vent length were seen along with mortality and unpleasant symptoms (Ose *et al*. 2019).

Reproductive Toxicity

Reproduction is critical aspect of all life forms for preserving ecological equilibrium. Environmental pollution exposure has havoc effects on the reproductive endocrine system andmay contribute to the decline in fertility (Chen *et al*., 2016; Wang *et al*., 2019). Pyriproxyfen did not cause any abnormality in developing CNS stem cells (Dzieciolowska *et al*., 2017). Truong *et a*l. 2016 discovered the developmental toxicity at high concentration in zebra fish. In rats no developmental defects or premature deaths were found (Koyama *et al*., 1989). Specific estrogenic action of pyriproxyfen was revealed in an in vitro system (Manabe *et al*., 2006).

The endocrine disrupting properties of pyriproxyfen impacts on variety of reproductive and ecological characteristics in daphnia(Ginjupalli *et al*., 2015; Kakaley *et al*., 2017; Chłopecka *et al.,* 2018; Tanaka *et al*., 2018; Watanabe *et al*., 2018). In male mice it impairs spermatogenesis and testicular architecture (Shahid *et al,* 2019). Prior research on the effects of pyriproxyfen on rats and dogs found a reduction in weight of seminal vesicle, prostate, and androgen sensitive organ.

It hinders human sexual and reproductive development. Diet, age, gender and profession contribute to reproductive system dysfunction. Human fetus, infants (long-term impacts on intellectual function) and children indicates greater vulnerability than adults (Birnbaum *et al*., 2003). Insuffient data available on the pyriproxyfen induced reproductive toxicity in rats. There is no report about its harmful effects on physiological, biochemical, and histopathological parameters. The objective of present study is to apply a biochemical technique to scrutinize the lethal consequences of pyriproxyfen on reproductive system of adult male Sprague Dawley rats

The effects of pyriproxyfen on HPG axis

The HPG axis is key biomarker of hormonal alterations (Lee *et al*., 2018). The gonadotropin hormones involved in synthesis of sex hormone LH and FSH (Qiu *et al*., 2019). The pyriproxyfen exposure decreased the expression of lhβ and fshβ genes. In brain aromatase cyp19b regulates the endogenous estrogen biosynthesis.

The expression of cyp19b and erα was considerably up-regulated in the brain of zebra fish in exposure to pyriproxyfen by 0.97 and 0.54-fold respectively at 100 µ g/L, indicates the estrogenic potential of pyriproxyfen. A higher level of circulatory E2 may be the cause of the enhanced brain aromatase cyp19b expression. (Xu *et al*., 2017). Transcriptional elevation in erα demonstrates that pyriproxyfen has estrogenic actions and impaired the reproductive system. Its erα mediated estrogenic activity in rat pituitary tumor cell line was reported (Manabe *et al*., 2006). The declined expression of lhβ and testosterone concentration also examined.

Aims and Objectives

Pyriproxyfen has harmful effects on male rat reproductive system; the current study was created to assess these consequences.

The aims and objectives are:

- To understand Leydig cells ability in pyriproxyfen treated adults Sprague Dawley rats by measuring plasma testosterone level.
- To evaluate stress response generated by pyriproxyfen exposure in the adults male Dawley rats by assessing plasma cortisol levels.
- To measure the toxic effect of pyriproxyfen on lipid profile and plasma protein levels.

Material and Method

The current study was directed in Laboratory of Reproductive Physiology, Zoology Department, Quaid-i-Azam University Islamabad. The resident ethical committee of Zoology Department, which is focused on managing in animals for research provided the rules for handling of animals.

Chemical used

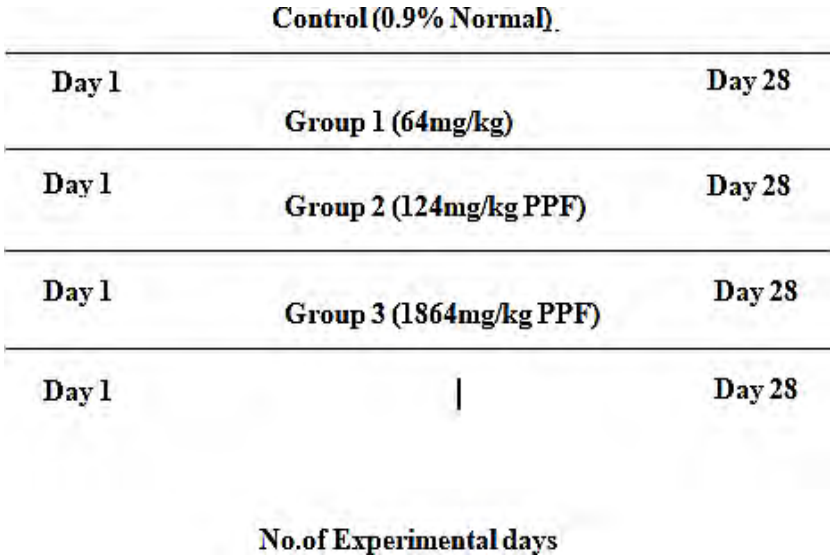
Chemical used Pyriproxyfen was purchased from ANQA AGRO

Animals

From animal house of Zoology Department Quaid-i-Azam University Islamabad, Pakistan, twenty adult male Sprague Dawley weighing an average of 150±10g were chosen. They were kept randomly in 4 groups and confined in separate stainless-steel cages (Ullah et al. 2016). During the experimentation, these animals were kept alive for 28 days in aeriform room having dark/light cycle lasts for 12 hour and 20 -26˚C temperature. During trial the animals were given Food chaw and tap water.

Experimental Design

Animals (n=5) were divided into 4 groups. All the doses were administered orally between 10-11am. For about 28 consecutive days, as shown in figure given below



Group 1

This group was considered as 'control group 'and treated with 0.9% normal saline.

Group 2

Animal received 62mg/kg of Pyriproxyfen dissolved in distilled water.

Group 3

124mg/kg dissolved in distilled water administered to rats.

Group 4

186mg/kg dissolved in distilled water delivered to animals

Final body weight of all animals was assessed and animals were sacrificed by decapitation on 29^{th} day

Blood and Tissue collection

For 28 days, the experiment was conducted. Animals were weighed and decapitated on 29th day. Following decapitation, heparinized syringes were used to collect trunk blood

directly and kept in heparinized tubes. For 15 minutes blood samples were centrifuged at 3000 rpm Plasma was isolated and stored at -20˚C. From all animals both testicular and epididymal tissues were obtained. Histological studies were done on epididymis and testicular tissue (right) secure in 10 % formalin.

Daily sperm production (DSP)

The prior to the homogenization process the previously frozen testicular tissues were allowed to thaw at room temperature for a short period of time. Spermatids that were resistant to homogenization (19^{th} stage of homogenization) were counted via a method described by Robb et al., 1978; tunica albuginea and parenchyma was weighted. The mixture then homogenized for 30 seconds in 5ml 0f normal saline containing 0.5% triton X-100. 5-fold dilution was done. In Neubauer chamber 20µl of sample was placed and under microscope at × 400 late spermatids' counting was done. In each sample average number of spermatids was counted three times. Number of spermatids per gram was measured to determine the overall sperm count per testis. Numbers of spermatids were resistance to homogenization divided by 6.3 to obtain number of days the spermatids remained in seminiferous epithelium

Formula for daily sperm production

Y=X/10 × 100 ×5×20×1000

Y= represents the number of spermatids in the homogenate

10= the number of observed squares in single reading

X= spermatids counted in Neubauer chambers

100 = total number of squares in chamber

5 = attenuation made with saline

20µl = homogenate for stacking chamber

1000 = converting µl into ml

DSP = Y/6.3

Competence of sperms = DSP/weight of de-capsulated testis)

Determination of glucose level in blood

Glucose in blood drawn from tail of rats was noted using glucometer on day 1st, 14th, 28th.

Total protein

Protein kit bought from AMEDA Labordiagnostik GmbH, Krenngasse, Graz, Austria (Cat # BR5202-S was used to assess total protein concentration in testicular tissues. Total protein was calculated by plotting the absorbance of kit standards against the absorbance of samples. It was measured in mg g−1 of tissue.

Procedure

- Samples, standard and blank were added into respective wells.
- Then added given reagent in each well one by one.
- For 10 minutes gently mix and incubated at 37°C.
- Absorbance of the sample and standard at 540nm within 60 minutes measured.
- Calculated the concentration was using the following formula.

A sample /A standard ×C standard = g/dl Total protein

Biochemical Analysis

Hormonal Plasma was collected and kept at 20°C. The frozen samples were thawed and used to study the effects of pyriproxyfen on triglycerides, cholesterol and HDL as parameters of Lipid profile.

Lipid profile

Contributing factor to an individual's total lipid profile is blood cholesterol and its associated lipoprotein varieties, HDL and Triglycerides. Triglycerides and cholesterol are fats and are necessary for cell health, but they can be harmful when they accumulate in blood. They can sometimes cause atherosclerosis. Blood pressure and coronary heart disease risk has been found to be strongly related to blood cholesterol level (cortan et al, 1999).

Lipid profile test

It is series of tests to determine the amount of cholesterol and other fats in blood. It measures these facts:

Triglycerides

LDL

Total cholesterol

HDL

Serum biochemistry analysis

Using a chemistry analyzer (AMP diagnostic) and AMP diagnostic kits (AMEDA Labordiagnostik GmbH, Austria) Low-density lipoprotein (Cat # BR3302) TC (Cat # REF10498999318389), High-density lipoprotein (Cat # 104989993194) and TG (Cat # REFBR4501) were measured in blood plasma.

HDL

HDL is termed as good cholesterol because it removes LDL from body, which reduces the risks of heart diseases. 40 to 60 mg/dL denotes healthy levels of HDL (Rizvi & Nagra, 2014).

Total cholesterol

It measures the total cholesterol in body. In adults it should be less than 200mg/dL.

Triglycerides

Your body converts unwanted calories into triglycerides which later stored in fat cells. High levels of triglycerides are present in obese people/animals.

Sixty mg/dL or above is considered the protective level.

Principle

Lipoprotein lipase (LPL) enzymatically hydrolyzes plasma triglyceride into glycerol and free fatty acids (FFA). In the presence of glycerol kinase (GK), Adenosine triphosphate (ATP) phosphorylates glycerol to adenosine diphosphate (ADP) and glycerol-3- phosphate (G-3-P) and Hydrogen peroxide and dihydroxyacetone phosphate (DHAP) produced by oxidation of G-3-P. Combination of phenol and 4- aminoantipyrine (4-AA) with hydrogen

peroxide (H_2O_2) under action of peroxidase (POD) secrete a red chromogem that is inversely proportional to the content of triglyceride in the sample.

$$\text{Triglyceride} + 3H^2O \rightarrow 3FFA + \text{glycerol}$$

$$ATPGK + \text{Glycerol} \xrightarrow{GK} ADP + \text{glyverol} - 3 - p$$

$$\text{Glyverol} - 3 - p + O2GPO \xrightarrow{GPO} H2O2 + DHA$$

$$4 - \text{phenol} + 4 - AA \xrightarrow{H202,} H,O + \text{Quinoneimine}$$

This substance can be used manually (see method below) and on most analysers applications are available on request.

Bring reagent samples and standard to room temperature.

Read against reagent blank.

Pipette as following

Table 01: shows amount of chemicals for Triglycerides

	Blank	Sample	Standard
Reagent	1.0*m*L	1.0*mL*	1.0mL
Sample	----	10µL	----
Standard	----	----	10µL

Gently mixed

Incubate at 37°C for five minutes

Absorbance of sample and standard observed.

If shielded from light color remain consistent for at 1 hour.

Cholesterol

Principle

Enzymatic based colorimetric technique with cholesterol esterase and cholesterol oxide oxidase was used.

$$\text{Cholesteryl esters} + H^2O \xrightarrow{CHE} \text{fatty acids} + \text{cholesterol}$$

$$O^2 + \text{Cholesterol} \xrightarrow{CHO} H^2O + \text{cholest} - 4 - \text{en} - 3 - \text{one}$$

$$\text{Phenol} + 2H2O2 + \text{4-aminoantipyrine} \rightarrow \text{quinonimine dye-4-en-3-one} + H^2O$$

(Red coloured)

Linear relationship is present between cholesterol concentration and color intensity.

Procedure:

These chemicals can be utilized in a number of automatic analyzers as well as manual assay.

Manual Procedure

Wavelength	500nm (Hg-546nm
Temperature	20-25°C /37°C
Cuvette	1cm

Pipette into the cuvette

Table 2: Shows the concentration of chemicals for cholesterol

	Reagent blank (RB)	Test	Standard
Cholesterol	1000µL	1000µL	1000µL

Bring up to the temperature of determination. Then Add

Standard	**----**	**----**	10µL
Sample	**----**	10µL	**----**

Incubate for 5 min at 37°C. Against reagent blank read the absorbance of the test and standard.

HDL

Principle:

There are two steps in distmel reaction assay:

(1)By using cholesterol esterase, oxidized cholesterol and then catalase chylomicron VLDL and LDL are eliminated.

Cholesterol

Cholesteryl ester → cholesterol + fatty acids

Cholesterol oxidase

O^2 + Cholesterol → cholesterol + H202

Catalase

2H202 → H2O + 02

2) After detergents in 2-Reagent have released HDL-Cholesterol, HDL-Cholesterol measurement was done. Catalase is inhibited by sodium azide in the 2-Reagent.

Cholesterol esterase

Cholesterol ester → fatty acids + cholesterol

Cholesterol oxidase

O^2 + Cholesterol → H202 + cholesterol

Permidase

2H2O2 → qumonc pigment + 4 H2O

Procedure

Wavelength	620nm, 630nm, 670nm,
Temperature	37°C
Optical path	1cm
Measurement	Against reagent blank

Substrate start

Table No: 03 shows the chemicals for HDL

	RB	Test	Cal
Reagent 1	300	300	300
Sample		5	
Calibrator	-	-	5
Mix well, incubate 5min at 37°C, and then add			
Reagent 2	100	100	100

Mix well; incubate calibrator after 5 minutes absorbance of A (T) and calibrator A against the reagent blank

Hormonal Analysis

Quantitative Determination of Testosterone Concentration

Using enzyme immunoassay (EIA) kits (Bio check Inc, USA) testosterone

Concentrations were evaluated quantitatively. The assay works on the following principle

Principle of the Test:

The basic principle of testosterone ELISA lies in the Competitive method. Coat micro well plates with goatanti-rabbit to form solid-phase antibodies. Add and HRP –testosterone, testosterone calibrator and antibody, secondary antibody is formed. The binding of HRP-testosterone is reversely proportional to testosterone concentration in serum. Remove the

Unbound testosterone-HRP. Add substrate (Chromogen A and Chromogen B) and detect absorbent value. Calculate testosterone content of samples through plotting concentration - absorbent value curve.

Procedure:

•Micro titration strips were marked that used. All the calibrator and controls were set duplicate.

•Added 50µl of calibrators, controls, and samples into respective wells then added 50µl of HRP conjugate and 50µl of antibody to each well one by one.

•Then covered the strips with a plate sealer. Mix the microliter plate gently. Incubated the plate at 37°C for about one hour. After this washed each well 3 times for about 10 seconds.

•Then added 50µl of Chromogen A and Chromogen b to each well one by one. Then again covered the strips and mixed the plate gently and for 60 minutes incubated at 37°C. To each well added 50µl stop solution and mix completely.

• Within 10 minutes absorbance of the plate noted.

Quantitative Determination of Cortisol Concentration

Using enzyme immunoassay (EIA) kits (Bio check Inc, USA) cortisol concentrations were evaluated quantitatively. The assay works on the following principle:

Principle of Test:

The basic principle of Cortisol ELISA lies in the competitive method. Working solution of anti-cortisol-biotin and cortisol-HRP conjugate was added to streptavidin coated wells. Cortisol enzyme (HRP) conjugate and cortisol have competitive binding. By washing buffer unbound cortisol and cortisol enzyme conjugate removed. Color intensity is inversely proportional to amount of cortisol in illustration.

Procedure:

Before beginning the experiment Reagents are allowed to stand at room temperature. Inserted the holder with specified the number of coated strips.

- 25µl of cortisol standard, control, and sera were added. After this added 50µl of biotin reagent and 100µl of cortisol enzyme conjugate to each well, one by one then mixed for about 10 seconds and incubation for about 60 minutes at room temperature were done.
    - 300µl of wash buffer was used for washing. After addition of 100µl of TMB substrate for 15 minutes incubated at room temperature.
    - Add 50µl stop solution to all wells and mixed.
    - Within 20 minutes absorbance at 450 nm noted.

Statistical Analysis

The variance to all observed data One-way ANOVA and Tukey's test used to examine. All findings were presented as Mean SEM. Graph Pad 5 was the Programme utilized for this analysis. The threshold for significance was established at p 0.005

Results

Body weight

At day 1, 14 and 28 Comparing all groups to the control no discernible difference observed while group 3 at day 14 and 28 showed highly significant fall (p=0.000) in body weight. At day 14 and 28 in comparison to group 1, group 2 showed non- significant change in body weight. While group 3 showed significant (p=001) decrease when compared with group 2 at day 14 and non-significant decrease at day 28. At day 28 comparison of group 3 with group 2 showed highly non-significant reduction in body waight.

Table 4: Mean ± SEM body weight (g) among adult male rats in control and treated groups

Parameter Body eight(g)	Control	Group 1	Group 2	Group 3	p- value statistics
Day 1	155.49±1.89	146.48±1.05	143.48±1.2	138.87±1.2	0.376
Day 14	146.48±0.88	141.87±1.61	136.40±1.2	130.59±1.5[a] ***b**	0.000
Day 28	141.00±1.48	136.79±1.40	134.49±`1.5	127.62±1.7 a***	0.003

Values are specified as Mean ±SEM. *, **, *** showing significance variance at p< 0.05, p<0.01, p<0.001 respectively. a represents value vs control, b indicates value vs group 1and csignifies value vs group 2.

Testis weight

Comparison to control no remarkable change in testicular weight was seen in group 1 and 2 while a very significant (p<0.001) reduction in testicular tissue was noticed in group 3. Non-significant change (p=0.378) in testicular weights demonstrated when group 2 compared with group 1. Significant drop (p 0.001) was found.in group 3 testicular weights wax compared to group 2,

Epididymis weights

The weight of the right and left epididymis did not differ significantly (p=0.274) between groups 1 and 2 and the control however, the right epididymis did differ significantly (p0.05) between group 1 and 3. A negligible decrease in right and left epididymal weight was observed among groups 2 and 3 when compared with group 1. A non-significant decrease (p=0.07) in epididymal weight was seen in group 3 in comparison with group.

Accessory organ weight

None of the group showed notable difference in weight of prostate (p=0.466) in comparison with control. As compared to control seminal vesicle weight did not differ significantly between groups while group 2 and 3 presented a notable change (p<0.05).

Table 5: Mean ± SEM testicular, Epididymis, and Accessory Organ weights after 28 days of exposure among adult male rats.

Parameters	Control	Group 1	Group 2	Group 3	P-value Statistics
Testis weights (R)	3.45 ± 0.00	3.46 ± 0.01	3.46 ± 0.01	3.42 ± 0.01 a***b***c***	0.000
Testis weights (L)	3.47 ± 0.01	3.46 ± 0.01	3.44 ± 0.01 a***	3.39 ± 0.00 a***b***c***	0.000
Epididymis weights(R)	0.54 ± 0.02	0.52 ± 0.02	0.47 ± 0.03	0.45 ± 0.02a*	0.074
Epididymis weights(L)	0.52 ± 0.04	0.51 ± 0.03	0.48 ± 0.02	0.44 ± 0.02	0.287
Prostate weights(g)	0.58 ± 0.04	0.56 ± 0.05	0.52 ± 0.07	0.48 ± 0.02	0.466
Seminal vesicle(g)	0.76 ± 0.04	0.72 ± 0.07	0.62 ± 0.04 a*	0.58 ± 0.02 a**b*	0.051

Values are specified as Mean ±SEM. *, **, *** showing significance variance at p< 0.05, p<0.01, p<0.001 respectively. a represents value vs control, b indicates value vs group 1and csignifies value vs group 2.

Sperm parameters

A significant to highly significant decline in sperm motility (%) was observed in all treated group compared to control group. Group 2 showed slight change as compared to group1 while group 3 exhibit non-significant changes compared to group1and group 2.As compared to control group 1 showed non-significant decline in sperm viability while group 2 and 3 showed highly significant fall (p=0.00).In comparison to group 1, group 2 and 3 showed non-significant fall while group 3 showed a highly significant fall in sperm viability (%) as compared to group2 (p=0.000). A non-significant decline in DSP was observed in group 1while group 2 and group 3 showed significant falls as compared to control. Non-significant changes observed in group 2 and 3 as compared to group 1.Group 3 showed non- significant declines in DSP when compared with group 2

Table 6: Mean ± SEM motility (%), viability (%) and DSP in control and treated adult male rats after 28 days oftreatment.

Parameters	Control	Group 1	Group 2	Group 3	p-value statistics
Sperm motility (%)	62.2±4.07	52.3±2.59^{a**}	45.30±2.59^{a***b*}	43.2±2.51^{a***}	0.000
Sperm viability (%)	82.2±2.46	72.2±4.07	64.30±2.49^{a***}	55.3±2.51^{a***c***}	0.000
Daily sperm production×10^{6}\Testis	2.23±4.47	1.69±1.33	1.11±8.54^{a**}	1.02±^{a**}	0.002

Values are specified as Mean ±SEM. *, **, *** showing significance variance at p< 0.05,p<0.01, p<0.001 respectively. a represents value vs control, b indicates value vs group 1and csignifies value vs group 2.

Glucose Level

A very remarkable decrease (p<0.001) assessed in blood glucose levels among all trial groups as compared to control. At day 14, in comparison to group 3 an important decline (p<0.01) noted in group 1 and group 2. At day 28 no measureable change was assessed in group 1and 2 while group 3 exhibits a noticeable fall.

Table 7: Mean ± SEM Glucose levels after 28 days of treatment among adult male rats

	Day	Control	Group 1	Group 2	Group 3	p-value statistics
Glucose level (mg/dl)	1	108 ± 0.71	105.6 ± 0.81 ^{a***b***c***}	101.8 ± 0.86 ^{a***b***c***}	94.40 ± 1.12 ^{a***b***c***}	0.000
	14	108.6 ± 1.21	103.4 ± 0.40 ^{a***}	100 ± 0.89 ^{a***b**}	92.40 ± 1.28 ^{a***b***c***}	0.000
	28	109 ± 1.22	102.8 ± 0.58 ^{a***}	98.4 ± 0.67 ^{a***}	90.60 ± 0.24 ^{a***b***c***}	0.000

Values are specified as Mean ±SEM. *, **, *** showing significance variance at p< 0.05,p<0.01, p<0.001 respectively. a represents value vs control, b indicates value vs group 1and csignifies value vs group 2.

Total protein

No significant change in total protein levels among all groups in comparison to control. In group 2 and group 3, comparison with group 1 no measureable alteration was observed. Group 3 showed non- significant increase in protein when compared with group 2.

Table 8: Mean ± SEM total protein contents (mg/g) in rats of control and treated groups after 28 days.

Parameter	Control	Group 1	Group 2	Group 3	P-value statistics
Total protein (mg0.5-1)	44.26 ± 0.67	48.86 ± 1.27	53.58 ± 0.85	58.01 ± 1.67 ^{b*}	0.003

Values are specified as Mean ±SEM. *, **, *** showing significance variance at p< 0.05,p<0.01, p<0.001 respectively. a represents value vs control, b indicates value vs group 1and csignifies value v

Cholesterol

Highly significant upsurge (p=0.000) in level of cholesterol in group 1, 2 and 3 was assessed when compared with control group. A very big uptick (P=0.000) in cholesterol levels was detected in group 2 and 3 in relation to group 1. Aremarkable increase was observed in group 3 (p=0.000) related to group 2.

Table 9: Mean ± SEM Cholesterol concentration (mg/dl) after 28 days of exposure among adult male rats.

Cholesterol (mg/dl)	Control	Group 1	Group 2	Group 3	p- value statistics
	142.43 ±3.9	255.82 ± 3.93^{a***}	274.95 ± 3.9^{a***b***}	297.21 ± 3.05^{a***b***c***}	0.000

Values specified as Mean ±SEM. *, **, *** showing significance variance at p< 0.05, p<0.01,p<0.001 respectively. a represents value vs control, b indicates value vs group 1and csignifies value vs group 2

Triglycerides

All treatment group animals had a triglyceride level that was significantly higher (p=0.000) than control rats. Highly substantial (p=0.002) change of triglycerides levels in group 2 and group 3 animals shown (p=0.000) when compared with group 1. When comparison to group 2, a non- significant (p=0.248) increase in triglyceride levels was found in group 3 animals.

Table 10: Mean ± SEM Triglycerides concentration (mg/dl) after 28 days of exposure among adult male rats.

Triglycerides (mg/dl)	Control	Group 1	Group2	Group 3	p- value statistics
	101.51± 4.59	160 ± 4.44^{a***}	179.62 ±4.62 ^{a***b*}	186.03 ± 0.46^{a***b***}	0.000

Values are specified as Mean ±SEM. *, **, *** showing significance variance at p< 0.05,p<0.01, p<0.001 respectively. a represents value vs control, b indicates value vs group 1and csignifies value vs group 2.

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LDL Cholesterol direct

In relation to control highly significant alteration (p=0.000) in LDL levels in group 1, 2and 3 was observed. Significant to highly-significant rise in LDL levels was seen in group 2 (p=0.001) and group 3 (p=0.001) as compared to group 1. Significant rise (p=0.05) was observed in LDL levels in group 3 in comparison to group 2.

Table 11: Mean ± SEM LDL Cholesterol direct concentration (mg/dl) after 28 days of exposure among adult male rats.

LDL (mg/dl)	Control	Group 1	Group 2	Group 3	p-value statistics
	47.04 ± 2.53	94.82± 5.45^{a***}	112.06 ±0.77^{a***b**}	121.17 ± 1.10^{a***b***c*}	0.000

Values are specified as Mean ±SEM. *, **, *** showing significance variance at p< 0.05,p<0.01, p<0.001 respectively. a represents value vs control, b indicates value vs group 1and csignifies value vs group 2.

HDL

There was no crucial decrease in levels of HDL in group 1 (p=0.132) while in group 2, 3 extremely significant (p<0.01; p=0.000) decline calculated. Group 2 showed measurable change (p=0.193) in HDL levels compared to group 1. Comparing group 3's HDL levels to group 1 resulted in a highly significant (p<0.01) drop. When group 3 animals were compared with group 2 results in no significant change (p=0.164).

Table 12: Mean ± SEM HDL Cholesterol direct concentration (mg/dl) after 28 days of exposure among adult male rats.

HDL (mg/dl)	Control	Group 1	Group 2	Group 3	p- value statistics
	64.91 ± 2.11	60.03 ± 1.37	55.88 ± 3.02^{a**}	51.42 ± 1.81^{a***b**}	0.003

Values are specified a s Mean ±SEM. *, **, *** showing significance variance at p< 0.05, p<0.01, p<0.001 respectively. A represents value vs control, b indicates value vs group 1and csignifies value v

Testosterone

A non-significant decrease in testosterone levels noticed in group 1, 2 and group 3 treated animals compared control. Group 2 and group 3 exhibit a non-significant decrease (p=0.604; p=0.719) in plasma levels of testosterone in comparison to group1 respectively. When group 3 treated animals were compared to group 2, the levels of testosterone were non-significantly lower (p=0.989).

Table 13: Mean ± SEM Testosterone concentration (ng/ml) after 28 days of exposure among adult male rats.

Testosterone (ng/ml)	Control	Group 1	Group 2	Group 3	p- value statistics
	4.83 ± 0.29	3.09 ± 0.25	2.62 ± 0.50	2.05 ± 0.68	0.264

Values are specified as Mean ±SEM. *, **, *** showing significance variance at p< 0.05,p<0.01, p<0.001 respectively. a represents value vs control, b indicates value vs group 1and csignifies value vs group 2.

Cortisol

A non-significant elevation in plasma cortisol levels noticed in group 1, 2 and group 3 treated animals compared control. Group 2 and group 3 exhibit a non-significant rise (p=0.247; p=0.573) in plasma levels of testosterone in comparison to group1 respectively. When group 3 treated animals were compared to group 2, the levels of testosterone were non-significantly lower (p=0.540).

Table 14: Mean ± SEM cortisol concentration (ng/ml) after 28 days of exposure among adult male rats.

Parameter	Control group	Group 1	Group 2	Group 3	p- value statistics
Cortisol (ng/ml)	71.9 ± 4.41	112.1 ±1.27	125. ± 4.42	134.08±1.44	0.021

Values are specified as Mean ±SEM. *, **, *** showing significance variance at p< 0.05, p<0.01, p<0.001 respectively. A represents Value vs control, b indicates Value vs group 1and c signifies Value vs. Group 2

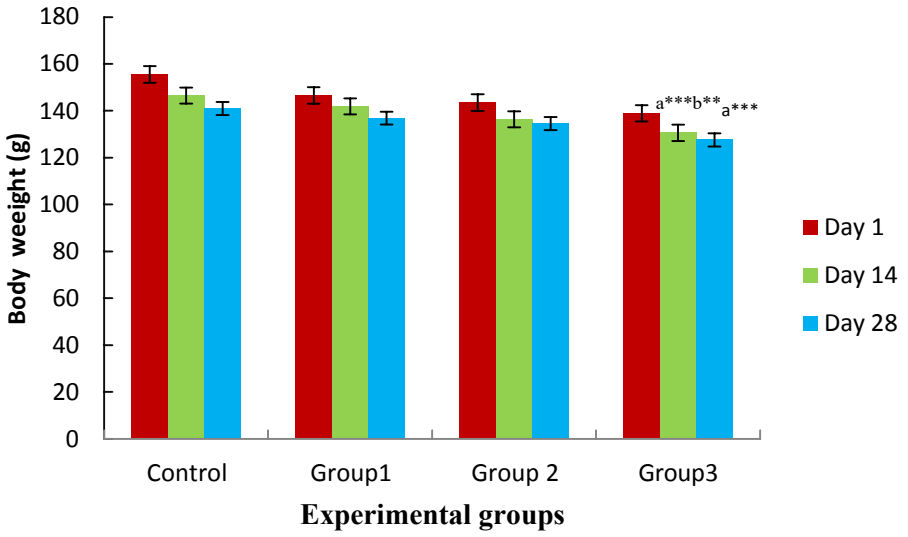

Figure 2: Mean ± SEM body weight (g) among adult male rats in control and treated groups

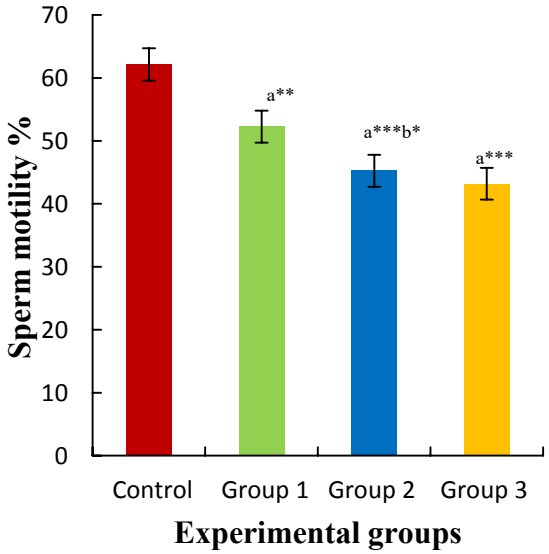

Figure 3: Mean ± SEM motility (%) in control and treated adult male rats after 28 days of treatment

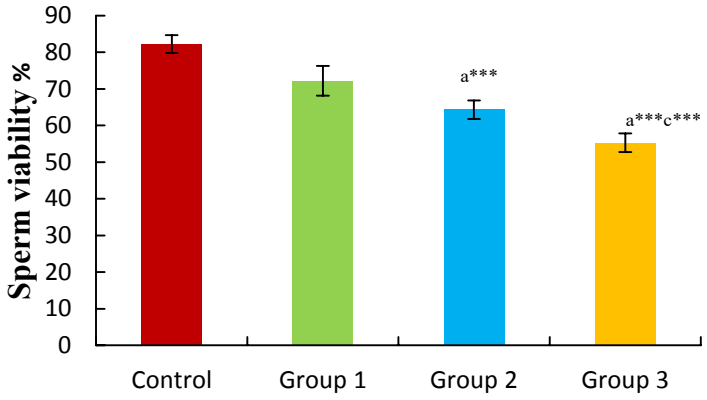

Figure 4: Mean ± SEM viability (%) in control and treated adult male rats after 28 days of treatment

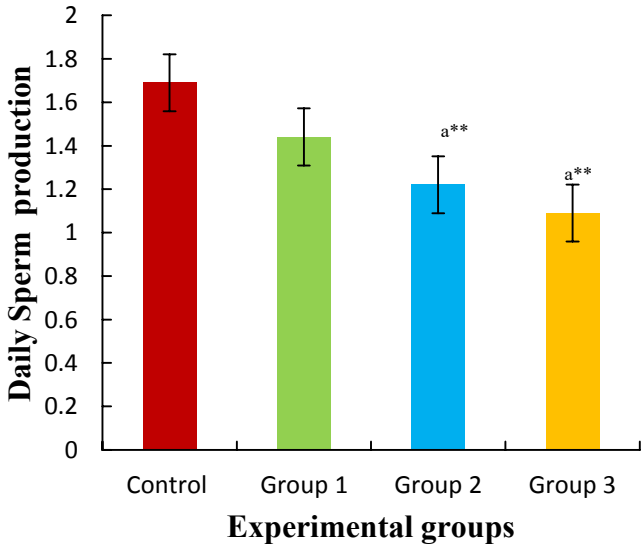

Figure 5: Mean ± SEM D S P in control and treated adult male rats after 28 days of treatment

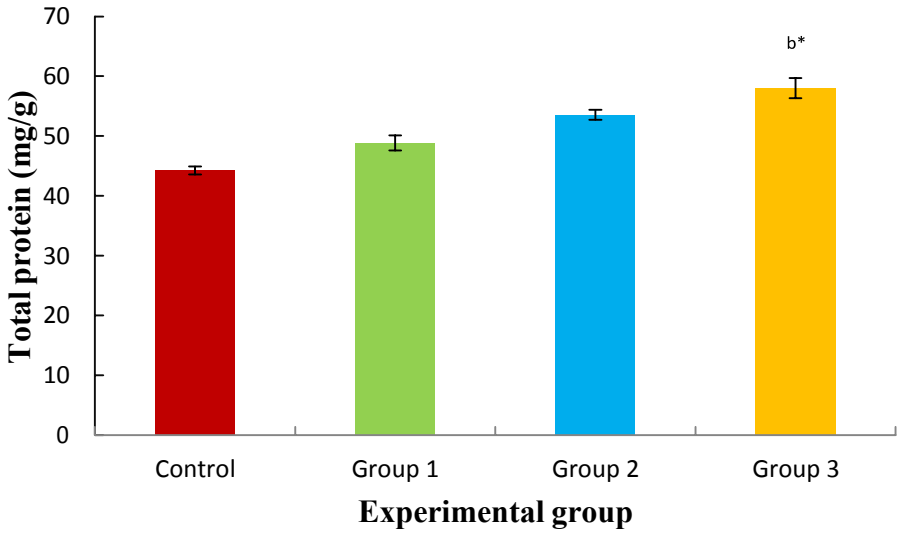

Figure 6: Total protein contents (mg/g) after 28 days of treatment in control and Pyriproxyfen treated groups

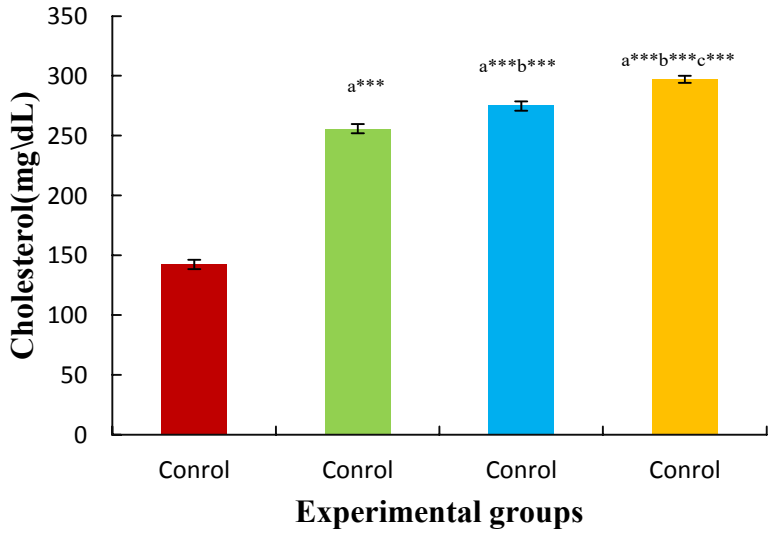

Figure 7: Mean ± SEM Cholesterol level (mg/dl) among adult male Sprague Dawley ratsfollowing treatment with Pyriproxyfen dosage.

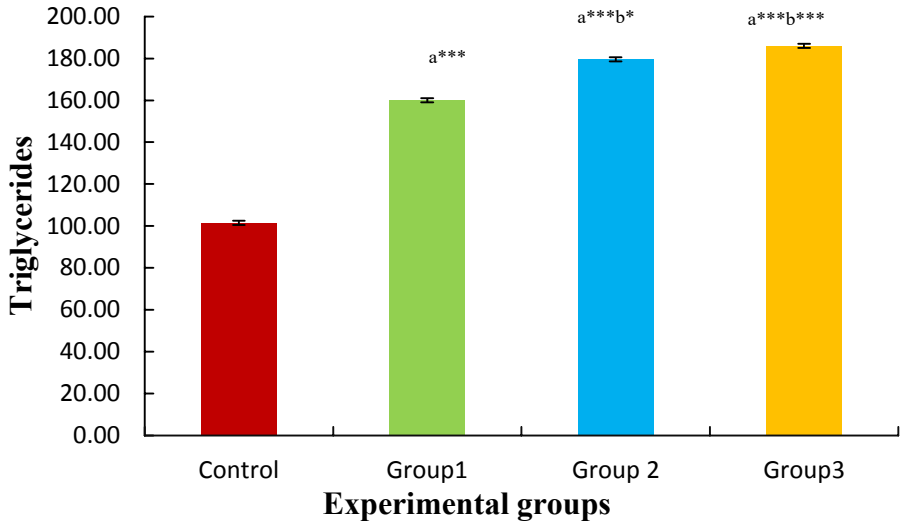

Figure 8: Mean ± SEM Triglycerides level (mg/dl) among adult male Sprague Dawley rats following treatment with Pyriproxyfen dosage

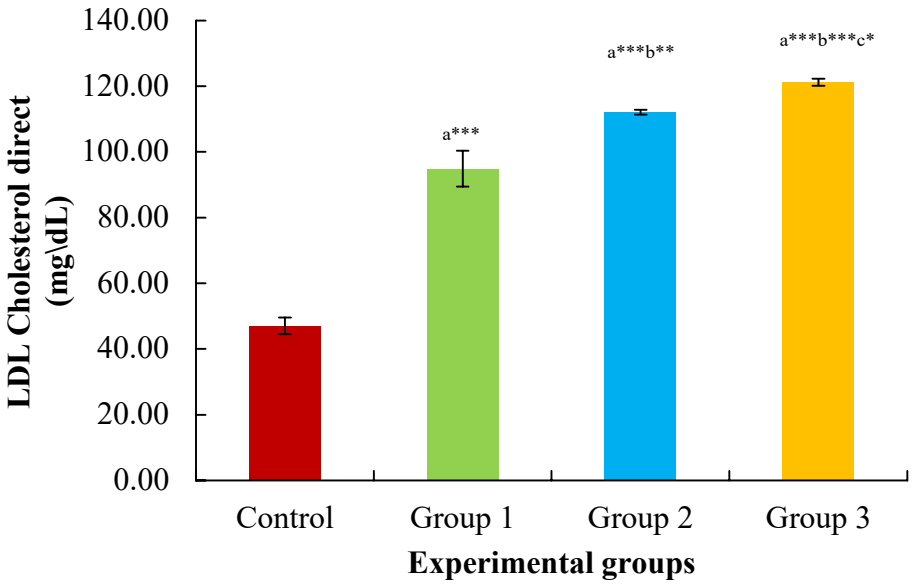

Figure 09: Mean ± SEM low- density lipoprotein, LDL level (mg/dl) among adult male Sprague Dawley rats following treatment with Pyriproxyfen dosage

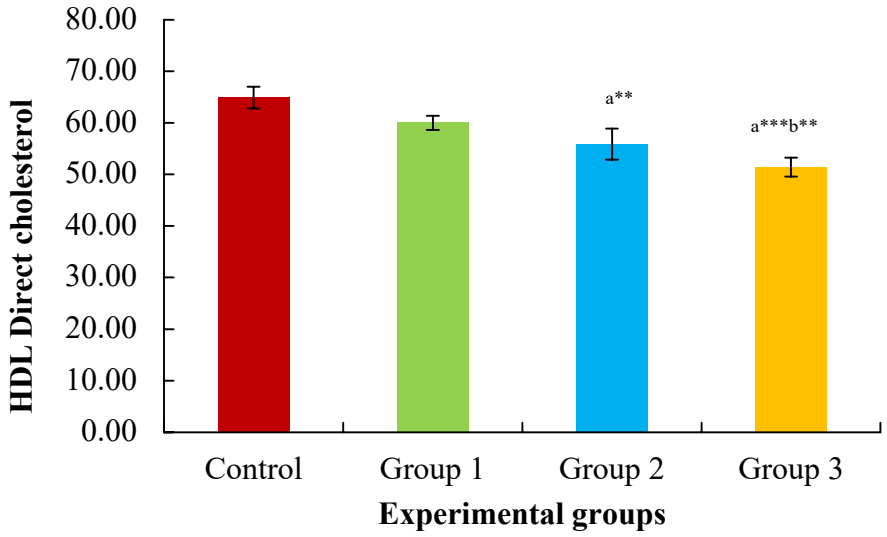

Figure 10: Mean ± SEM High- density lipoprotein, HDL level (mg/dl) among adult male Sprague Dawley rats following treatment with Pyriproxyfen dosage

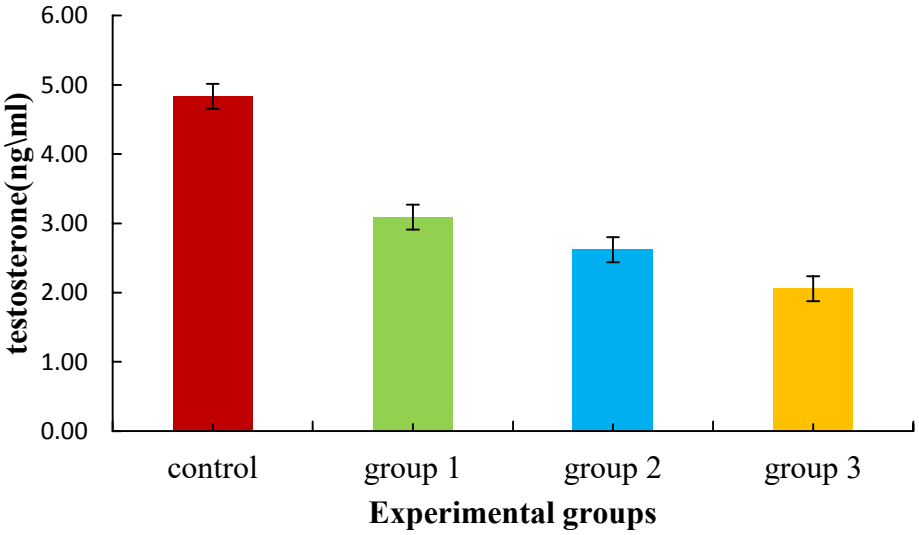

Figure 11: Comparison of plasma testosterone levels in treated and control groups after 28 days of treatment

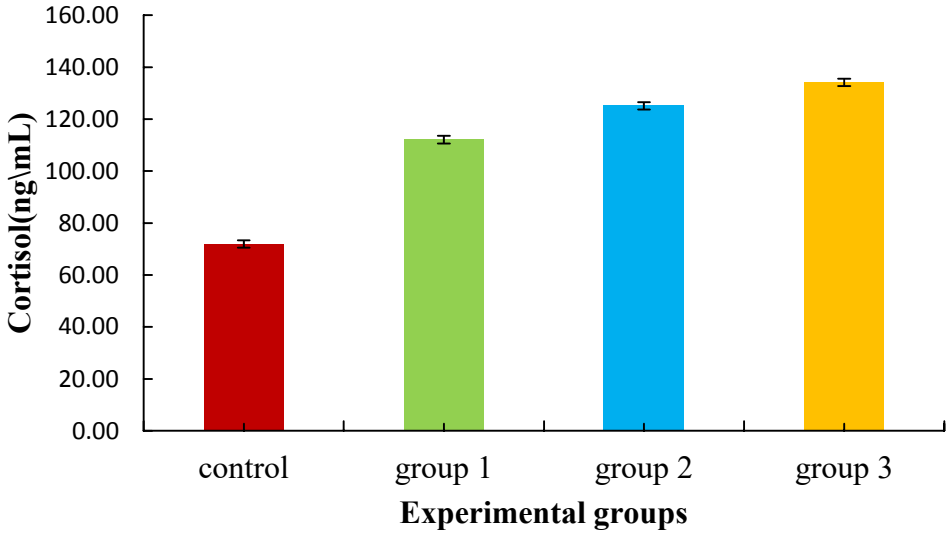

Figure 12: Comparison of plasma cortisol levels between control and pyriproxyfen treated groups

DISCUSSION

Endocrine disruptors are the substances that disturb the body's normal homeostasis via numerous mechanisms. They are classified into sets. This study is established to evaluate the toxic effects of one of the EDCs pyriproxyfen an insecticide. Application of pesticides and insecticides induces significant possibility of infertility and aberrant sperm counts in animals. Previous studies have inspected that they may induce adversative effects like cancer endocrine disruption and reproductive disorder. Their consistent use results in the contamination of the water resources and environment (Hussain *et al*., 2019; Yang *et al*., 2021). Pyriproxyfen is a juvenile hormone analogue insecticide (Fukushima *et al*., 2005; Jambulingam *et al*., 2008; Tsunoda *et al*., 2013; Lwetoijera *et al*., 2014). The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) did not declared pyriproxyfen as genotoxic and carcinogenic chemical. (Invest and Lucas, 2008). Pyriproxyfen causes the impairment of the reproductive system, altering spermatogenesis resulting in the apoptosis of Leydig cells and germ cell lining. In order to reduce the dangers of pyriproxyfen to the public health it is essential to examine its potential toxicity. The current study was intended to investigate reproductive toxicity of pyriproxyfen in adult male Sprague Dawley rats. Physical characteristics such as body weight, visceral tissue weight, and clinical conditions are regarded as credible toxicity biomarkers for toxicological evaluation. As part of safety evaluation in this study no death was observed during 28 days period of administration. In clinical observation, alopecia observed in late period of administration, loose stools, decreased food consumption and increase in water consumption was observed. Body mass index (BMI) is a tool used to evaluate obesity and to monitor changes in body weight, thus it is useful for assessing the efficiency of weight loss therapy (Knutsen *et al*. 2017). The findings of this study suggested that the body weights of all treatment groups did not significantly reduced, however in group 3 there was significant decrease in body weight which is assumed to be connected to food avoidance because of drug toxicity. Treatment caused decrease in body weight similar to previous studies (Koyama *et al*., 1989). Organs and relative organ weights are employed as indicators for organ toxicity in toxicological research. Similar to the earlier study in which pyriproxyfen caused a drop in the weight of testis and accessory organs, the current investigation revealed a significant decrease in testis weight of rats (Koyama *et al*., 1989). The testis weight is dependent on the reduction in seminiferous tubules size and number of differentiated

spermatocytes. The weight of the testis tends to decrease as the population of germ cells is reduced (Sanchez *et al*., 2004). Low serum testosterone concentration might be the cause of the decrease of accessory organ weigh. Testosterone preserves the structural integrity and functional activity of accessory sex organs (Moor *et al*., 1930). The groups treated with pyriproxyfen showed a substantial decrease in DSP which is similar to the previous study, decrease in sperm concentration, motility, increase in abnormal sperm rates and depletion of sperm count was seen due to drug toxicity; (Shahid *et al* .,2019). In our study in treated groups highly significant (0.000) decrease in blood glucose levels was seen in comparison to control group which is similar to previous findings (Koyama *et al*., 1989). Pyriproxyfen could prevent the feeding of rats and decrease of some biochemical compounds like glucose could be due to starvation stress. Various serum biochemical parameters used to evaluate harmful effects of environmental pollutants and pathophysiological rank of terrestrial and aquatic animals (Hussein *et al*., 2021). Rats given pyriproxyfen have also been shown to have harmful toxic effects on serum indicators (Koyama *et al.,* 1989). In our experiment significant increase in serum protein level of pyriproxyfen administered groups was observed these results are similar with previous findings (Koyama *et al*., 1989). In the current trial, serum biomarkers, including cholesterol, triglycerides levels significantly increased in all treated groups of rats when compared with control and these findings are according to the previous study (Koyama *et al*., 1989). In our experiment serum triglycerides LDL was increased highly significantly by pyriproxyfen treatment, these results support earlier research showing that taking pyriproxyfen increased the amount of triglycerides. (Naseem et *al*., 2022). Administration of pyriproxyfen produces more ROS. High ROS production affects the lipids (triglycerides, cholesterol, HDL) (RADak *et al*., 1999). In cells and body organs lipids play crucial structural and functional roles (Rawi *et al*., 2012). Dyslipidemia caused by high levels of ROS. Oxidative stress cause imbalance in serum lipid concentration in the circulation (Martins *et al.,* 2018). The cell's susceptibility to oxidative stress is severely damaged by the decline in antioxidant levels, which compromises its capacity to detoxify ROS's effects and results in an increase in lipid profile. (Pérez *et al*., 2009). HDL is defined as good cholesterol Higher the level of HDL healthier the animal is due to exposure of pyriproxyfen which cause the oxidative stress and disturb the normal body homeostasis. In our experiment serum HDL level decreases and no previous study is present on effect of pyriproxyfen on level of HDL. Sex hormone testing is one of the most comprehensive and practical indicators of

reproductive damage. (Ji *et al*., 2013b). In this study testosterone level decreased significantly in pyriproxyfen treated groups as linked to control and these outcomes are consistent with the earlier research. (Maharajan *et al*., 2020).High level of ROS might decrease the testosterone level. During spermatogenesis testosterone regulates libido and promotes spermatid elongation (Dutta *et al*., 2013). According to the results of the prior evaluations, cortisol levels considerably rise in treatment groups in our study compared to control groups. Cortisol is critical for maintaining homeostasis; alterations in various physiological parameters may result from disproportion in the HPG axis. (Bennion et al., 2015; Späth-Schwalbe et al., 1991; De Quervain et al., 2017).

Conclusion

The results of current research point out that male adult Sprague Dawley rats when exposed to differential doses of pyriproxyfen experience severe oxidative stress and testicular toxicity. Administration of pyriproxyfen triggered a marked decline in body, testis and accessory organ weights. Pyriproxyfen interferes with spermatogenesis in seminiferous tubules. Plasma protein level increased significantly whereas cholesterol, LDL and triglycerides levels were significantly increased. There was marked increase in cortisol levels while plasma testosterone levels reduced significantly. More studies using different animal models are required to better understand how pyriproxyfen affects oxidative stress and testicular injury.

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