A biochemical approach to evaluate the ameliorative effects of *Adiantum capillus-veneris* L. against furan induced toxicity in adult male Sprague Dawley rats.

By

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FACULTY OF BIOLOGICAL SCIENCES
QUAID-I-AZAM UNIVERSITY ISLAMABAD
2022

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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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SPECIAL THANKS

'WHAT WE DO FOR OURSELVES DIES WITH US, WHAT WE DO FOR OTHER REMAINS AND ARE IMMORTAL.'

(ALBERT PIKE)

I PAY MY SPECIAL THANKS TO MY SUPERVISOR **DR. SARWAT JAHAN** FOR HER KIND GUIDANCE, HELP AND VALUABLE ASSISTANCE.

AND

I PAY MY SPECIAL THANKS TO MY MOTHER"S, **MAMO WALI, MAMO SAJJID, MAMO NAZAR** FOR THEIR COUNTLESS LOVE, CARE, ENCOURAGEMENT, AND SUPPORT THROUGHOUT MY LIFE.

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.

Rimsha Javed

CERTIFICATE

This dissertation submitted "A biochemical approach to evaluate the ameliorative effects of *Adiantum capillus-veneris L*. against furan induced toxicity in adult male Sprague Dawley rats." **Rimsha Javed** is accepted in its present form by the Department of Zoology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirements for the degree of Master of Philosophy in Reproductive Physiology.

Supervisor: _____

Dr. Sarwat Jahan

External Examiner: _____

Chairman: _____

Dated:

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All praises are to Almighty ***Allah****, who is Light upon light, who guides to His light whomever He wills. Many Thanks to him as he blessed us with the Holy prophet* ***Hazrat Muhammad****, peace be upon him, for whom the whole universe is created and who enabled us to worship only one God. He brought us out of darkness and enlightened the way of heaven. I am thankful to Almighty* ***Allah*** *for giving me the strength and honour to complete this research work successfully.*

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May Allah bless all those who love me (Ameen)

Rimsha Javed

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LIST OF ABBREVIATIONS

AR	Androgen Receptor
ALT	Alanine aminotransferase
AST	Aspartate Aminotransferase
ALP	Alkaline Phosphatase
ACV	*Adiantum capillus-veneris* L
BDA	Butene1,4-dialdehyde
BMI	Body Mass Index
CAT	Catalase (CAT)
CNS	Central Nervous System
DSP	Daily Sperm Production
EIA	Enzyme Immunoassay
EDCs	Endocrine-Disrupting Chemicals
EPA	Environmental Protection Agency
EFSA	European Food Safety Authority
ER	Estrogen Receptors
FSH	Follicle Stimulating Hormone
FDA	Food and Drug Administration
GR	Glucocorticoid Receptor

GST	Glutathione -S-transferase
GnRH	Gonadotrophin Releasing hormone
HRP	Horse Reddish Peroxidase
HPG	Hypothalamus-Pituitary Gonadal axis
HPA	Hypothalamus-Pituitary adrenal axis
HPT	Hypothalamus-Pituitary Thyroid axis
IARC	International Agency for Research on Cancer
LDL	Low-Density Lipoprotein
LH	Luteinizing Hormone
PCBs	Polychlorinated Biphenyls
RXR	Retinoid X Receptor
ROS	Reactive Oxygen Species
TC	Total Cholesterol
TG	Triglycerides
TMB	Tetramethylbenzidine
WHO	World Health Organization

Abstract

Furan (C_4H_4O) is a highly volatile organic heterocyclic molecule that has been classified as a probable human carcinogen. Recently, it has been discovered in many heat-treated foods such as baby foods, sauces, vegetables, soups, and is known to induce oxidative stress in animals. A remarkable number of modern drugs have been developed and isolated from plant sources that are known to be effective against chemicals that act as endocrine disruptors. Because of the therapeutic and nutritional value of *Adiantum capillus-veneris* L., it has been used as herbal medicine. Therefore, the goal of the current study was to determine ameliorative effects of *Adiantum capillus- veneris* L. against furan-induced toxicity in adult Sprague Dawley male rats. To conduct a present study, adult male Sprague Dawley rats were separated into 4 groups (n=5); Group I was given orally with 0.9% normal saline, while Group II was orally administrated with 40mg/kg of furan, Group 3 was orally administered with 250mg/kg methanolic leaf extract of *Adiantum capillus-veneris* L., and Group 4 was orally administrated with furan and ACV for 28 consecutive days, respectively. On the 29th day, animals were weighed, and decapitated; and tissues (testis and epididymis) and blood sample were collected for sperm parameters and biochemical analysis. Plasma was separated and stored at -20˚C for biochemical and hormonal analysis. The present study showed a remarkable decrease ($p<0.001$) in daily sperm production and percentage of viability and motility of sperm in furan group in comparison to control group. Analysis of lipid profile indicated an increase ($p<0.001$) in levels of cholesterol, low-density lipoprotein (LDL), and triglycerides, and a decrease in levels of high-density lipoprotein (HDL) in the furan administered group. The findings of hormonal analysis depicted a reduction ($p<0.001$) in testosterone levels, while increase ($p<0.001$) in cortisol concentrations were evident in furan-treated groups. For all the studied parameters, *Adiantum capillus-veneris* L. showed ameliorative effects than that of furan. Conclusively, plant *Adiantum capillus-veneris* L. ameliorate toxic effect of furan on reproductive system and protect the testes. Therefore, it is suggested that due to the protective effect of plant *Adiantum capillus-veneris* L. in male rats, it can be used to restore the toxic actions mediated by endocrine disruptors.

Introduction

Recently, the focus of scientific literature has increased on endocrine-disrupting chemicals (EDCs) and their importance in human pathophysiology. EDCs are the exogenous chemicals substance that disturbs hormonal activities or causes disruption in the hormonal pathway (Gore *et al*., 2015; Thomas Zoeller *et al*., 2012). Three types of actions have been shown by EDCs (1) endocrine action; (2) pathologic endocrine mediated-action; (3) endocrine association action (Alexander *et al*., 2013; Slama *et al*., 2016). EDCs are the artificial exogenous chemicals that regulates genomic expression, and also interfere with the endocrine system as proposed by the European Food Safety Authority (EFSA) (Zama & Uzumcu, 2010). EDCs have been grouped into five main categories: **industrial** includes polychlorinated dioxins, polychlorinated biphenyls (PCBs), and alkylphenol, **agricultural** such as insecticides, weed killer, insect killer, antifungal agents, **households** such as phthalates, polybrominated biphen, bisphenol A, and **drugs** and **heavy metals** that include lead, cadmium, mercury, and arsenic and food-based acrylamide and furan (De Coster & Van Larebeke, 2012; Kabir *et al*., 2015b; Monneret, 2017). EDCs have contaminated the human body through various pathways. Common exposure pathways are inhalation, ingestion, and direct contact via skin. EDCs move into the food chain and accumulate in animals and humans body by using various pathways (Balaguer *et al*., 2017; Dickerson & Gore, 2007; Kabir *et al*., 2015b).

EDCs are highly lipophilic and possess a long half-life, hence, accumulated in the adipose tissues (Schug *et al*., 2011; Thomas *et al*., 2012; Disruptors, 2015). EDCs bind and activate different hormone receptors such as glucocorticoid receptor (GR), androgen receptor (AR), thyroid hormone receptor (TR), estrogen-related receptor (ERR), aryl hydrocarbon receptor (AhR), estrogen receptor (ER), retinoid X receptor, and also mimic actions of natural hormones (Balaguer *et al*., 2017; Heindel *et al*., 2015; Monneret, 2017). EDCs may also impair the production, transportation, metabolism, and removal of hormones, lowering concentrations of endogenous hormones (Thomas *et al*., 2012). EDCs have additive or synergistic effects on the endocrine system (Barouki, 2017; Nohynek *et al*., 2013). The actions of EDCs are not confined to particular axis or organ, the different axes of the body are the target of EDCs including the hypothalamus-pituitary-adrenal axis, hypothalamus-pituitary-thyroid axis, hypothalamus-pituitary-gonadal axis (Thomas *et al*.,

2012). Evidence shows that the central nervous system (CNS) may also be affected by EDCs, mediated by improper functioning of the hypothalamus and pituitary gland, which may affect normal activities of peripheral glands with unknown effects (De Coster & Van Larebeke, 2012; Kabir *et al*., 2015a). EDCs may altering adipose tissue, disrupting endocrine regulation of adipose tissue and synthesis of adipocytokine, reduced basal metabolic rate, and modifying the hunger and satiety control (Street *et al*., 2018). EDCs can affect behavior in both males and females in different mammals including humans. Even at very low doses, through epigenetic changes EDCs change the normal steroidogenesis pathway in males, and also affect the development of rodent brain (Masuo & Ishido, 2011).

The EDCs can mimic sex gonadal hormones and can bind to estrogen receptor (ERs) , which can interfere with the signaling of hormonal because the reproduction is affected by EDC's actions (Monneret, 2017). The risk of breast cancer may increase following exposure to, polychlorinated biphenyls (PCBs), polychlorinated dioxins, cadmium, furans, and ethylene oxide. Heavy metals and pesticide exposure appear to increase the risk of prostate carcinogenesis (De Coster & Van Larebeke, 2012).

Recent scientific literature showed that EDCs exposure during the development period not only damages the living organisms but also affects future generations (Lauretta *et al*., 2019).

Heat-induced food toxicants:

Thermal processing is used to preserve food, especially in the formation of shelf-stable foods with certain nutritional qualities. On the other hand, it results in the formation of heat-induced toxic compounds, known as thermal process pollutants, which have toxicological effects, and pose health risks to humans. Acrylamide, chloropropanols, and furan are well-known as thermal process chemicals in foods (Maga & Katz, 2009; Tareke *et al*., 2002). Chloropropanols and furan were discovered in food in the 1970s, while acrylamide was discovered in 2002 (Mogol & Gö, 2016). The International Agency for Research on Cancer has classified furan as a probable human carcinogen (*IARC* 1995*).*

Furan:

Furan (C_4H_4O) is a highly volatile organic heterocyclic molecule. Furan has been classified as a probable human carcinogen by the National Toxicology Program (NTP) and US Department of Health and Human Services and the international agency for Research on Cancer (IARC) (*IARC* 1995*;* NTP 1995). In year-long gavage research, furan even at low doses induced cancer in various experimental animal species, including rodents (Batool *et al*., 2021). Furan and furan-contained chemicals was first detected in meals. In 1983, presence of furan in volatile components of coffee was seen and after few years it was confirmed (Merritt *et al*., 2002). Previous literature reported that the furan was present in various foodstuffs such as chicken, bread, and tinned beef. Initially, breakdown of carbohydrate is closely linked with furan formation (Maga & Katz, 2009).

Recently, furan has been recorded in many heat treated foods, including vegetables, baby foods, sauces, canned and jarred, and soups (US FDA 2004). Carbohydrates and amino acids are present in coffee, which break down at higher roasting temperatures and react with one another by different mechanisms resulting in generation of furan to greater extents (Rahn & Yeretzian, 2019). Recent literature reported the occurrence of furan in coffees that are available easily in markets (Becalski *et al*., 2016; Chaichi *et al*., 2014). As a result, the existence of furan in various items such as baby foods cereals and coffee may has a considerable impact on overall human body to furan exposure, necessitating a greater understanding of the health concerns linked with their intake (Rahn & Yeretzian, 2019).

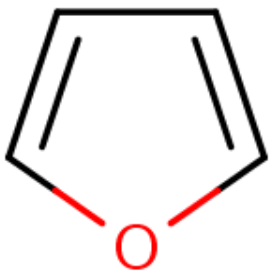

Figure 01: Chemical structure of Furan (Rahn & Yeretzian, 2019)

Furan Formation:

Baking, roasting, pasteurizing, cooking, and sterilization are thermally driven processes that involve furan formation (Crews *et al*., 2007). Because of its carcinogenicity and presence in many heat treated foods, such as meat products, coffees, and cereal, has gotten a lot of attention around the world (Altaki *et al*., 2017; EFSA, 2010). Absorption of furan through the gastrointestinal tract and its breakdown by P450 2E1 hepatic cytochrome mediated ring-opening, which leads to the formation of cis-2-butene-1,4-diol, which is a highly reactive metabolite that can react with amino and thiol groups in glutathione and other peptides (Chen *et al*., 1995; Lu *et al*., 2010). The thermal degradation of carbohydrates and ascorbic acid and its derivatives, as well as thermal oxidation of polyunsaturated fatty acids, have been considered as potential precursors and alternate pathways for the production of furan in meals, rather than a single mechanism (Crews & Castle, 2007). Nevertheless, mechanisms of their formation remain unclear. Perez-Locas and Yaylayan (2004) suggested that ascorbic acid has the greatest potential for producing furan, followed by some sugar/amino acids mixtures; reaction conditions, such as temperature, time, and pH, can also have a significant impact on furan formation (Fan *et al*., 2008) (Figure 02).

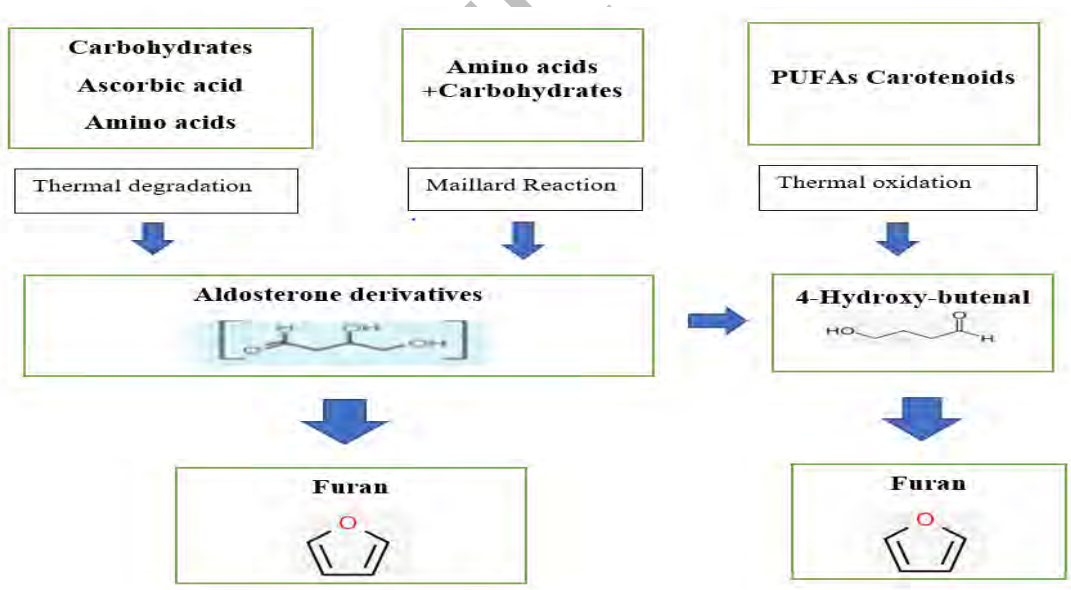

Figure 02: Synthesis of furan from various precursors such as carbohydrates, amino acids, and polyunsaturated fatty acids through different pathways.

Exposure to furan:

Human food contains thousands of physically different chemical substances, the majority of which are of natural origin and components that are added such as colorants, nutrients, and flavors. Furan levels were found to be higher in nutritious drinks and bakery products (Katrine Knutsen *et al*., 2017; FDA, 2004).

The concentration of furan content in various food substances was used to determine human exposure to furan. Furan exposure is increased because of presence of furan in baby products and overcooked food day by day. Previous literature showed that the presence of furan in human urine morning samples in various concentrations (Sudjarwo *et al*., 2017). Adult humans in developed countries are exposed to furan at a rate of 0.34 to 1.23 g/kg BW/day averaging 0.78 g/kg BW/day (EFSA, 2010). The average daily exposure at the age of 3-12 months is 0.27 to 1.01 g/kg BW/day (Altaki *et al*., 2017). 0.23-1.77 g/kg BW/day of furan level was reported in the newborn (Minorczyk *et al*., 2011)**.** Coffee is the most abundant source of exposure in adults (Altaki *et al*., 2017). Breastfeeding or formula feeding is a main source of furan toxicity in infants (Lehmann *et al*., 2018)**.** High levels of furan were observed in baby carrot juices, prune juices, and flavors of nutritional drinks (Wegener & López-Sánchez, 2010)**.** Recent research showed that bottled and canned drinks are a significant source of furan for newborns (Wegener & López-Sánchez, 2010) **(Figure 03).**

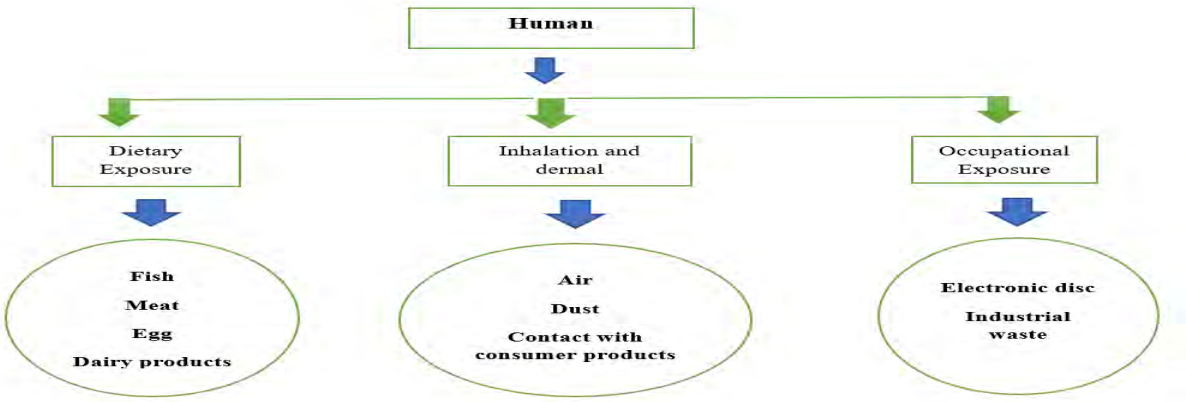

Figure 03: Different routes of furan exposure

Toxicology of furan

Recent observations showed that furan induces several types of cancer. Humans and different animals biological system are also affected by furan exposure (Hamadeh *et al*.,

2004; Uçar & Pandir, 2017; *IARC* 1995). Furan has been shown to have negative effects on rodent's vital organs (Ldeniz *et al*., 2011; Webster *et al*., 2014). An elevation of serum alanine aminotransferase (ALT) is closely linked to hepatic necrosis, caused by furan exposure in rats (Baş *et al*., 2016; Gill *et al*., 2010). In rats, exposure to furan increases levels of malondialdehyde by reducing levels of glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) (Baş *et al*., 2016). Furan altered the histology, ovarian cell's DNA structure, levels of malondialdehyde, and activities of antioxidant enzymes (Uçar & Pandir, 2017). Furan also caused DNA damage by generating reactive oxygen species in lymphocytes and sperm (Pandır, 2015). Some studies showed that furan has antiandrogenic action (Cooke *et al*., 2010; Karacaoĝlu & Selmanoĝlu, 2010). Recent clinical literature showed that the mother's breastmilk contained a higher level of furan and dioxin than 11-week-old babies (Pluim *et al*., 1993). Furan exposure via breastfeeding or food, has been linked to different fetal abnormalities due to its ability to pass through the placental barrier (Krowke *et al*., 1990). Decrease in levels of testosterone and decrease in viability and motility of sperm and included cell death in germ and Leydig cells after exposure to furan in adult male rats was reported by Cooke *et al*., 2014, Karacaoğlu *et al*., 2010).

Oxidative stress caused by furan:

When antioxidant levels are lower, excessive generation of reactive oxygen species (ROS) is harmful (Embola *et al*., 2002). Excessive ROS generation induced toxicity in the reproductive system of different mammals, including humans. Oxidative damage can cause abnormal sperm morphology, as well as decreased sperm motility and egg production (Aitken *et al*., 2011; Iwasaki & Gagnon, 1992; Mazzilli *et al*., 2014; Sukcharoen *et al*., 1996). ROS production increases lipid peroxidase, which changes acrosome reaction, sperm DNA damage, and mucogenic capacity (Ichikawa *et al*., 1999). Excessive ROS damages gonadotrophins receptors, lowers cyclic AMP and inhibits steroid secretion by affecting corpus luteal (Aten *et al*., 1992). In the presence of unsaturated fatty acids in the membrane, spermatozoa are affected by oxidative stress (Aitken *et al*., 2011). Mitochondria are found in spermatozoa and provide a consistent supply of energy. Excessive ROS generation affects inner and outer mitochondrial membranes and releases cytochrome C, which triggers apoptosis (Thuillier *et al*., 2003). Antioxidant enzyme protects living cells by reducing ROS formation as well as by

detoxifying ROS by hydrogen peroxide conversion and dismutation while catalase converts hydrogen peroxide to water (Imlay *et al*., 1988; Wiseman & Halliwell, 1996). Contaminated elements in the human body usually target the liver because the breakdown of chemicals occurred in the liver which eventually leads to hepatocyte destruction and liver toxicity (Livingstone, 1991; Patel *et al*., 2012). Furan is quickly absorbed in the body and can also easily pass through biological membranes due to its low polarity (Bakhiya & Appel, 2010; Crews *et al*., 2007).

Previous research reported that the primary targets of furan toxicity are kidney and liver. As the main part of the defense mechanism of the living cell, catalase (CAT) converts ROS to hydrogen peroxide into water, then water protects the cells from ROS toxicity (Syed *et al*., 2012). Furan was found to be toxic to the hepatocytes, pancreas, and renal cells. Furan is harmful to the pancreas by exerting oxidative stress (El-Habiby *et al*., 2017). Epigenetic changes are linked to the effect of furan (Conti *et al*., 2014)

Endocrine-disrupting potentials of furan

Food and Drug Administration (FDA) previously recognized that food-based contaminants, furan and acrylamide, have the potential for endocrine disruption (Robin and Clanci, 2007; FDA, 2004) The normal production of steroid hormones is linked to the development of male reproductive organs, such as the prostate, Wolffian ducts, epididymis, seminal vesicles, and vas deferens but food-based contaminants interact with these hormones and have a negative effect on their target organ development and functioning (Cooke *et al*., 2014). Food-based contaminants block the enzymes 5α-reductase and aromatase, which are responsible for converting androgens to testosterone. Food-based contaminants exposure disrupts estrogen receptors (ERα and ERβ) expression levels. ERβ receptor expression in humans is sustained through morphogenesis and cellular differentiation but declines throughout early puberty. Increased levels of plasma androgens also boost the expression of the receptor associated with benign prostate hyperplasia and prostate cancer. The development stages of animals can also be affected by furan exposure. When animals are exposed to food-based contaminants during the stage of organ development, everlasting changes occur (Richter *et al*., 2007).

Furan exposure disturbs the hormonal profile of species. Furan can influence behaviors and sex-specific development by disturbing the hypothalamic-pituitary gonads (HPG)

axis, which is involved in the production of GnRH that contribute to reproductive maturation, and the hypothalamic-pituitary-adrenal axis, a stress mediator (Adewale *et al*., 2009; Schug *et al*., 2011). The adrenal cortex also secretes the hormone cortisol which further activates the HPA axis (Schreier *et al*., 2015). It has been observed that both chemical and non-chemical stressors disrupt the HPA axis (Cory-Slechta *et al*., 2008; Miller *et al*., 2007).

There are to suggest that furan has a negative impact on hypothalamic and pituitary functions (Gore *et al*., 2015; Kabir *et al*., 2015b). Furan interacts with the HPA-axis, during steroidogenesis (Martinez-Arguelles & Papadopoulos, 2015). Cortisol that is released by the HPA and HPI axes is a stress indicator in invertebrates (Barton *et al*., 2002; Heisler *et al*., 2007). The elevated level of cortisol increased the energetic costs, which affects the process of metabolism, reproduction, neurogenesis, and immune function, hypothalamus-pituitary thyroid (HPT) and HPA axes are also involved in the regulation of reproductive functions as the HPA axis influence the performance of HPG axis during development (Bernier *et al*., 2004; Dobson *et al*., 2007; Fitzpatrick *et al*., 2012; Hogan *et al*., 2004; Leal *et al*., 2011).

Reproductive toxicity of furan:

Contaminants, pesticides, and food additives are all known to be harmful to the reproductive system (Ijaz *et al*., 2021). Furan is one of the contaminants that may affect the reproductive system in male rats by lowering the levels of reproductive hormones (Cooke *et al*., 2014-b; Ijaz *et al*., 2021). Furan has also a significant impact on the testis, epididymis, and prostate gland (Luo *et al*., 2005; Ijaz *et al*., 2021).

Furan toxicity in the liver:

The main target organ of furan toxicity and carcinogenicity is the liver (Gill *et al*., 2010). In recent findings, different serum biochemical variables such as lipid profile and liver enzyme are typically used to determine liver damage before histopathology confirmation (Lopez-Lopez *et al*., 2021) Within the liver, bilirubin conjugated aspartate alkaline phosphatase (ALP), alanine aminotransferase (ALT), aminotransferase (AST). Total bilirubin is released into the circulation after cellular damage (Smith & Walker, 2002). Another complication of furan overdose is dyslipidaemia. The lipid metabolism take place in liver because liver is primary site for it and plays a significant part in serum

protein synthesis. Different diseases of the liver lower the levels of cholesterol, triglyceride, and high-density lipoprotein by limiting the liver's biosynthetic capacity and potency (Subhan *et al*., 2012).

Regardless of the toxicity of a specific organ, changes in levels of cholesterol, triglyceride, glucose, and serum proteins work as indicators of wide-ranging metabolic events (Smith, 2002). The mechanisms involved in these changes are unknown. Recent literature reported that increasing the intake of fatty acids foods as an energy source to balance the absence of carbohydrate resources may take part in lowering the levels of triglycerides. Furthermore, hepatocyte injury may increase a different amino acids levels in the liver (Ramm *et al*., 2016).

Furan Mitigation strategies:

On regular basis, humans are exposed to furan through different furan containing foods, and it is essential to assess the health risks associated with furan exposure. The primary source of (40-80%) dietary furan exposure is coffee in adults (Arisseto *et al*., 2011). However, mitigating furan is still a difficult task and there are currently no commercially available solutions (Anese & Suman, 2013). The various pathways involved in furan formation and the importance of techniques used for food safety and boosting aroma and flavor, as well as intricate interaction between furan retention and food matrix in food are the primary reason for this (Arisseto *et al*., 2011). However, no commercial technique is still available for furan mitigation (Anese & Suman, 2013).

***Adiantum capillus-veneris* Linn**

Plants and their products have been used since ancient times for food and shelter, and to cure different ailments (D'cruz *et al*., 2010). Traditionally, folk medicine is being used by many people around the world (Lucinda *et al*., 2010). A number of modern drugs have also been developed and isolated from plant sources (Mustapha, 2013). The earliest type of healthcare known to mankind is herbal medicine. Throughout history, herbs had been used by all societies. In the evolution of modern civilization, it was an important step. With period, each community added to its knowledge base about the medicinal power of herbs in their area. Herbal medications made up a large part of today's pharmaceuticals. According to World Health Organization (WHO), herbal medicine is used a major segment of the world's population (80 percent) currently for some part of basic health care.

Because of the therapeutic and nutritional value of *Adiantum capillus-veneris* L. was used as herbal medicine (Al-Snafi, 2015)

Adiantum, a genus of the Adiantaceae family, is found worldwide, from hot tropical zones to cool temperate zones. Sumbul and Hansraj are the local names of *Adiantum capillus-veneris* L. in Pakistan (Al-Hiyasat *et al*., 2002). Chemical composition of maidenhair fern has, flavonoids, steroids, tannins, terpenoids, and alkaloids (Khodaie *et al*., 2015). The anti-inflammatory, analgesic, wound healing, anti-fungal, anti-diarrheal, anti-bacterial, anti-diabetic, antioxidant, anti-spasmodic, diuretic, and anti-urolithiasis detoxifying agent and hypocholesterolemia properties of this plant roots, rhizomes, and the fronds of the plant species have been indicated (Dehdari *et al*., 2018). An anti-inflammatory effect of the ethanolic leaf extract of this plant against the toxic substances .71.15% anti-inflammatory effects were recorded at a 100 mg/kg dose of plant extract (Madboli & Seif, 2021). Due to the versatility of actions of *Adiantum capillus-veneris* L. plant extract is used against the toxic effects of furan.

Traditional uses:

Adiantum species were used for treatment of cough and cold, chest complaints, as an expectorant, to stimulate lactation, to help kidney function, and as dandruff and an antiparasitic. Antidandruff, laxative, refrigerant, demulcent, depurative, weak emmenagogue, emollient, weak expectorant, sudorific, galactagogue, antitussive, astringent, stimulant, and tonic were all employed on the fresh or dried leafy fronds. Tea was made from the dried fronds of the plants for the same purposes (Ahmed *et al*., 2013; Al-Snafi & Al-Snafi, 2015; Johnson & Sowrby, 1899)

Pharmacological effects

Anti-diabetic activity

The presence of flavonoids and tannins in methanolic leaf extract of *Adiantum capillus-veneris* L. has antidiabetic potentials (Ranjan *et al*., 2014). The plant's ability to increase weight is due to its ability to repair hepato-renal-damaged cells (Sultan *et al*., 2012). Furthermore, the species was found to have antihyperglycemic properties comparable to acarbose, as a reference drug (Kasabri *et al*., 2017).

Hypocholesterolemic effect

The methanolic leaf extract of *Adiantum capillus-veneris* L. has hypocholesterolemic potential by showing a great impact on levels of low-density lipoproteins and high-density lipoprotein and cholesterol (Al-Hallaq *et al*., 2015).

Anti-obesity effect

In vitro model exhibits that aerial parts of *Adiantum capillus-veneris* L. have a phospholipase inhibitory effect comparable to orlistat. The most responsible phytoconstituent is chlorogenic acid (Kasabri *et al*., 2017).

Anti-testosterone-induced hair loss effect

In a testosterone-induced alopecia model in mice, the effect of methanolic leaf extract of Maidenhair fern on hair growth was evaluated and resulted increase in follicular density (Noubarani *et al*., 2017).

Treatment of COVID-19.

Ancient medics employed plant extract used for treating different diseases. In modern medicine, *Adiantum capillus-veneris* L. also possesses pharmacological efficacy to treat many symptoms same to that caused by COVID-19 and underlying medical conditions (Hendawy, 2020).

Anti-fungal and anti-microbial activity

Adiantum capillus-veneris L. also contain phytoconstituents which are responsible for antifungal activity (Aspergillus Niger, Aspergillus terries, Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Saccharomyces cerevisiae, Fusarium sp., etc.). These phytoconstituents also have antimicrobial activities (Rajurkar *et al*.,2012).

Antioxidant property

Adiantum capillus-veneris L. exhibits antioxidant properties because flavonoids, reducing sugar, tannins and saponins, are present in leaves (Rajurkar *et al*., 2012).

Part used:

Adiantum capillus-veneris L. with fresh or dried leafy fronds, a dried herb with rhizome and roots are being used medicinally (Johnson & Sowrby, 1899).

Distribution in the world:

Adiantum capillus-veneris L. is found in places such as shady and moist all over world. It is found in Europe along the Atlantic coast as far as Ireland (Ahmed *et al*., 2013).

Distribution in Pakistan

Balakot, Changa Gali, Abbottabad (altitude 2,350 m) Mansehra, Battagram (altitude 1,100 m), Murree, Rawalpindi (altitude 1,800 m), and District Shangla are among the places where *Adiantum capillus-veneris* L. plant is found in Pakistan.

Topography:

On the globe, the Shangla district is located from 34° 31’ to 33° 08’ N and 72° 33’ to 73° 01’ E. The districts Bata gram and Kala Dhaka Springier are located on the east of district Shangla, district Swat is located on the west, district Buner is located on the south, and district Kohistan is located on the north. The forest covers 8,090 acres, with an average rainfall of 1778 mm while monthly is 150 mm, maximum snowfall of 60 inches from November to March, a maximum temperature is above 25°C in June and July, and a minimum up to 0°C in December. The plant name has been verified (http://www.theplantlist.org/) and the taxonomy of the plant according to National Plant Data Center, NRCS, USDA is as follows.

Kingdom: Plantae

Sub-Kingdom: Traciobionta

Division: Pteridophyta

Class: Filicopsida

Order: Polypodials

Family: Pteridaceae

Genus: adiantum. L

Species: *Adiantum capillus- veneris* Linn

***Adiantum capillus- veneris* Linn**

(Ahmed *et al* ., 2012)

Aim and Objectives

This study aims to investigate the protective effects of plant *Adiantum capillus-veneris* Linn. against toxicity caused by furan caused in adult male Sprague Dawley rats. The focus objective include :

- To evaluate testosterone levels in blood plasma.
- To measure stress level by cortisol.
- To evaluate lipid profile in blood plasma.
- To determine the effect on sperm parameters of testis.

Materials and Methods

The study was carried out at the Rep. Phy Lab, Department Zoology, QAU Islamabad. The ethical committee of the Department of Zoology approved guidelines and regulations for animal handling and management.

Chemical

Furan concentration (CAS 110-009, Sigma Aldrich Co. Ltd, Sigma -Aldrich Poole, Dorset,>99%pure) was prepared in corn oil on the day of use and stored in sealed brown bottles. Furan was administrated orally through gavage in corn oil at a dose of 40mg/kg for 28 days. 240mg/kg of methanolic leaf extract of *Adiantum capillus-veneris* L. was given. Based on previous findings of furan stability, doses were made separately in a volume-to-weight ratio (v: w) and stored in the refrigerator (sealed with plastic closure and modified silicon septa) for 14 days (NTP 1993). After 14 days, the same protocol was followed once more. Before being given to animals, furan was dissolved in corn oil, and plant extract was dissolved in normal saline.

Animals

Twenty adult Sprague Dawley male rats (Rattus norvegicus), weighing of 150±10g, were taken from Animal House QAU Islamabad. Rats were separated into four groups at random and kept individually. (Ullah *et al*., 2016) The Ethical Committee of the Zoology Department approved animal handling and experimental procedures.

Plant Material

Identification of the leaf sample of *Adiantum capillus-veneris* Linn was done by "Dr. Mushtaq Ahmad at Plant Systematics and Biodiversity Lab and Herbarium of Pakistan, Quaid-i-Azam University Islamabad". This plant was collected from agricultural and cultivated fields of Alpuria, Chakesar, Hayatabad, Shangla, Lilowni, Ajaori, Kass, and Shahpur, Pakistan.

Plant Extract Preparation

Adiantum capillus-veneris Linn leaves were removed from the stem and dried in air. The dried leaves about 2 kg were stored until the extract was prepared . The leaves were crushed and sieved through a Waring blender. By using methanol, leaves dried powder according to this ratio (leaves to solvent ratio 1:10) was extracted. Whatman filter paper is used for filtration of the plant extracts and concentrated on a rotary evaporator (Gulfraz *et al*., 2007).

Experimental Design

For the present study, the animals (n=20) were divided into four groups. All the doses were administrated orally between 10-11 am for consecutive 28 days.

Group I

This group was treated with 0.9% Nacl and considered a control group.

Group II

Group II rats were provided with 40mg/kg of furan, in corn oil.

Group III

Methanolic leaf extract dissolved in saline administration at a dose of 250mg/kg.

Group IV

Group IV animals were given 40mg/kg of furan dissolved in corn oil and 250 mg/kg of methanolic leaf extract dissolved in saline.

The final body weights of all the animals were recorded on day 29 and the animals were decapitated.

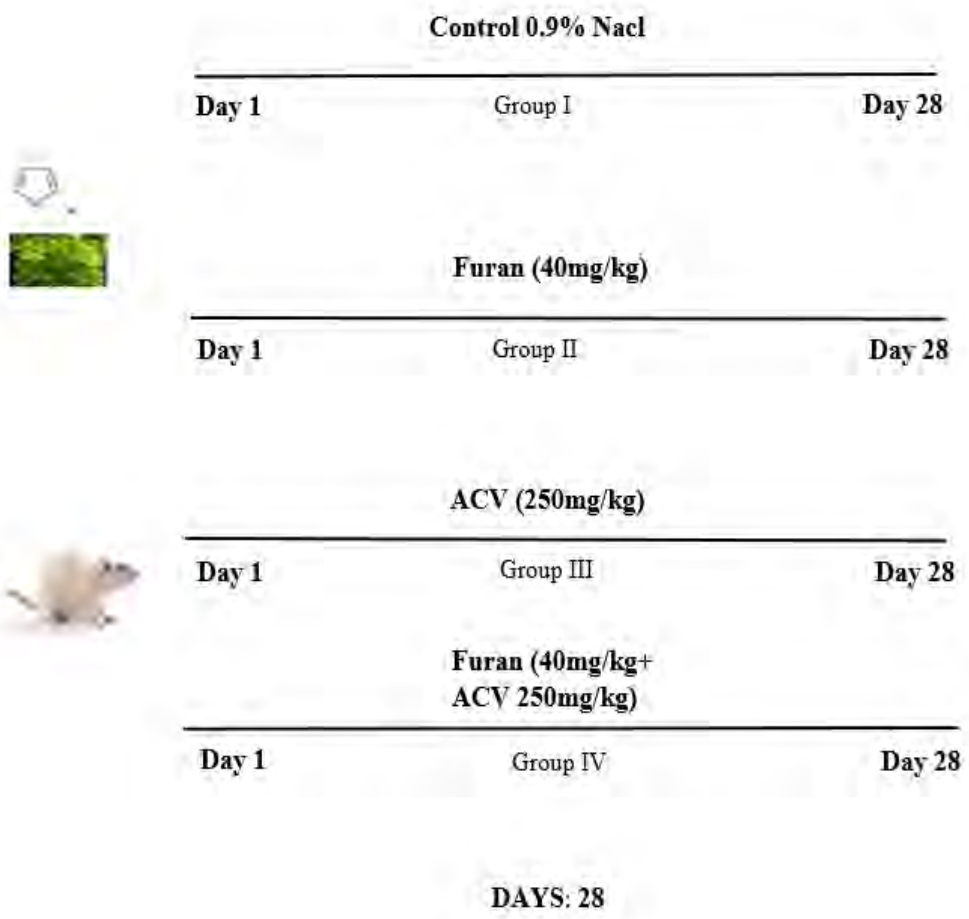

Figure 4: Experimental Design

Blood and Tissue collection

The experiment was carried out for 28 days. The animals were weighed and decapitated on day 29. Heparinized syringes were used to collect trunk blood directly after decapitation and kept in heparinized tubes. Blood samples were centrifuged at 3000 rpm for 15 minutes. Plasma was separated and kept at -20˚C till it came time to study it. Both testicular and epididymal tissues were obtained from all the animals. Analysis for daily sperm production, ice-cold saline was used for cleaning of left testis and was stored in the freezer. The viability and motility of sperm and epididymal sperm count was determined from the left epididymis after washed and weighed, then minced for more assessment. On testis and epididymis (right), the histological analysis was done that were fixed in 10% formalin.

Sperm Parameter:

Assessment of Sperm Motility

Small cauda portion of the epididymis was cut and placed in normal saline solution (1ml) at 37˚C and crushed for assessment of sperm motility. Homogenate sample (10µl) was placed on warmed slides by using pipette. A minimum of 10 fields were examined under high power microscopy (40× magnifications) and 100 spermatozoa were calculated (Halvaei *et al*., 2012).

Assessment of Sperm Viability

For determination of viability of sperm, and nigrosine-eosin test was used. The dye eosin-nigrosine of about (25µl) were mixed with samples of semen. Smear was prepared by placed 15µl of this mixture on a glass slide, and at room temperature slides was dried. Later, by using a light microscope (40× magnification) was used for the examination of these slides. The spermatozoa that remained unstained (white) are alive whereas stained red spermatozoa are dead. At least 100 sperm cells were counted and obtained the dead and alive spermatozoa percentage (Halvaei *et al*., 2012).

Daily Sperm Production (DSP)

At room temperature, thawed testis tissues were thawed for a few minutes before the homogenization process. Spermatids that were unaffected by homogenization (19th stage of spermiogenesis) in the homogenate, were counted by following the method of Robb *et*

al. (1978); Tunica albuginea was removed, and then parenchyma was weighed and homogenized for 30 seconds in 3ml of 0.9% NaCl containing 100 X 0.5% triton and then homogenized (Robb *et al*., 1978). The dilution of the resulting homogenate was made up to 5 folds. In Neubauer's chamber, 20µl of the sample was added and by using a microscope (x40 magnification), late spermatids were counted. To determine the spermatids number, for each sample at least three readings were taken. From this reading the spermatids total number per testis was calculated, then divide by weight of testis to get the spermatids number. That considered the capability of sperm production. The spermatids that were non-effective against homogenization process was divided by 6.3, for the calculation of DSP, which characterizes the spermatids stayed in the seminiferous epithelium for days.

Daily Sperm Production formula

Daily sperm production was calculated using formula Y = No. of sperms (N) ×25×1000×5×25* = Y/6.3

Were,

Y= No. of spermatids present in homogenate

N = Total No. of spermatids in Neubauer's chambers are counted.

25= Total No. of squares in the chamber.

5 = With physiological saline dilutions made.

1000 = to convert µl into ml

25*= dilutions made with PBS

Daily sperm production (DSP) = Y/6.3

Biochemical Analysis:

Serum biochemistry analysis

Using a chemistry analyzer (AMP diagnostic) and AMP diagnostic kits (AMEDA Labordiagnostik GmbH, Austria) and, TC (Cat # REF10498999318389), TG (Cat # REFBR4501), High-density lipoprotein (Cat # 104989993194), and Low-density lipoprotein (Cat # BR3302) were measured in blood plasma obtained from the experiment as given in handbook of instruction.

Hormonal Analysis

Quantitative Determination of Testosterone Concentration

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

Principle of the Test:

The basic principle of testosterone ELISA lies in the competitive method. Coat microwell plates with goat anti-rabbit to form solid-phase antibodies. Add testosterone antibody, testosterone calibrator, and HRP -testosterone to form a secondary antibody. The binding amount of HRP- testosterone is reversely proportional to testosterone content 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:

- Microtitration 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 microtiter 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 incubated at 37°C for about one hour. Finally added 50µl stop solution to each well and mix completely.
- Read the absorbance of the plate within 10 minutes.

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. To the wells coated with streptavidin, the samples, a working solution of the cortisol-HRP conjugate, and a solution of anti-cortisol-biotin are added. For binding sites, cortisol in the patient's serum competes with the cortisol enzyme (HRP) conjugate. Washing buffer removed unbound cortisol and cortisol enzyme conjugate., The concentration of cortisol is inversely proportional to the intensity of the color in samples upon addition of substrate. A standard curve linking color intensity to the concentration of cortisol was created.

Procedure:

Reagents are allowed to stand at room temperature, prior to starting of assay

Placed the desired number of coated strips into the holder.

- Then added 25µl of cortisol standard, control, and patient sera. 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. then added 100µl of TMB substrate and incubated for 15 minutes at room temperature.
- Finally added 50µl stop solution to all wells and mixed completely.
- Read the absorbance at 450 nm within 20 minutes.

Statistical Analysis

Graph pad Prism software was used to perform a one-way analysis of variance (ANOVA) followed by a Post-hoc Tukey's test to compare different groups. All the results were presented as Mean ± SEM. The significance level was set at $p < 0.05$.

Results

Bodyweight:

In comparison with the control group, the furan treated group had a highly significant decrease ($p<0.001$) in body weight. In ACV ($p=0.90$) and ACV +Furan ($p=0.41$) treatment groups, non-significant difference in body weight was seen in comparison with the control group. In comparison with the furan group, the ACV and ACV+ Furan treatment groups had a highly significant increase ($p<0.001$) in body weight. However, non-significantly decrease ($p=0.48$) was evident in the ACV+ Furan treated group in comparison with the ACV group (Table 1).

Table: 1 Mean ± SEM body weight gain (g) among adult Sprague Dawley male rats following treatment with furan and *Adiantum capillus-veneris* L. leaf extract.

Parameter	Control	Furan	ACV	Furan + ACV	P value statistics
Body weight gain (g)	41.17±4.38	21.09±3.02^{a***}	43.56±1.08^{b***}	42.06±4.21^{b**}	0.002

*p< 0.05, **p< 0.01, ***p< 0.001, values are stated as mean ± SEM showing significant variance respectively.

a value used in the comparison of control, while b value used in the comparison of furan and c value used in the comparison of ACV

Testicular weight:

When compared to the control group highly significant decrease ($p<0.001$) was observed in weight of both testis (right, left) in rats treated with furan. When compared with control group, non-significant increase in weight of both testis (right, left) among ACV ($p=0.70$) and Furan +ACV ($p=0.70$) treatment groups was observed. A significant increase ($p<0.05$) in weight of both testis (right, left) among ACV and Furan +ACV groups in comparison to the furan group. In Furan +ACV group, no significant change ($p=0.79$) was observed as compared to the furan group (Table 2).

Epididymis Weight:

A highly significant increase ($p<0.001$) in both right and left epididymal weight was seen in rats treated with furan, in comparison to control group. While, in comparison with control, a non-significantly increase among ACV and Furan+ ACV groups was noticed. However, no significant change ($p=0.60$) was observed in both right and left epididymal weight among ACV and Furan +ACV groups as compared to the furan group as well as the ACV group (Table 2).

Accessory Organs Weight:

When compared to control group, a remarkable decrease ($p<0.001$) in weight of prostate and seminal vesicle in rats treated with furan was observed. While non significantly increase ($p=0.59$) in prostate and seminal vesicle weight was evident among ACV and Furan +ACV groups as compared to the control group. In comparison to the furan group, a highly significant increase ($p<0.001$) in prostate and seminal vesicle weight was seen among ACV and Furan +ACV treated groups (Table 2).

Table:2 Mean ± SEM Testicular weights (g), Epididymis weights (g), and accessory organ weight (g) among adult male Sprague Dawley rats following treatment with furan and *Adiantum capillus-veneris* L. leaf extract.

Parameters	Control	Furan	ACV	Furan + ACV	P value statistics
Testis weight (R)	1.43±0.02	1.32±0.05^{a***}	1.45±0.02 ^{b*}	1.44±0.02 ^{b*}	0.009
Testis weight (L)	1.42±0.02	1.30±0.03^{a***}	1.43±0.05 ^{b*}	1.42±0.02 ^{b*}	0.02
Epididymis (R)	0.12±0.07	0.66±0.01^{a***}	0.71±0.06^{a***}	0.62±0.03^{a***}	0.001
Epididymis (L)	0.56±0.12	0.64±0.10^{a***}	0.70±0.05^{a***}	0.60±0.02^{a***}	0.68
Prostate gland	0.65±0.01	0.50±0.01^{a***}	0.67±0.01^{b***}	0.66±0.01^{b***}	0.001
Seminal Vesicle	0.74±0.01	0.40±0.03^{a***}	0.78±0.01^{b***}	0.77±0.02^{b***}	0.001

*p< 0.05, **p< 0.01, ***p< 0.001, values are stated as mean ± SEM showing significant variance respectively.

a value used in the comparison of control, while b value used in the comparison of furan and c value used in the comparison of ACV

Sperm Motility

In comparison with the control, remarkable decline in sperm motility (%) was evident in the furan group ($p<0.001$). Non-significant increase was evident in sperm motility (%) among ACV ($p=0.98$) and Furan+ ACV ($p=0.25$) groups in comparison with the control group. There was a significant increased ($p<0.01$) in sperm motility (%) among ACV and Furan+ ACV groups in comparison with the furan group. However, no significant change ($p=0.25$) in sperm motility (%) was observed in the Furan+ ACV group in comparison with the ACV group (Table 3).

Sperm Viability:

There was a prominent decrease in sperm viability (%) in the furan group ($p<0.001$) observed in comparison with the control group. No significant increase ($p=0.26$) was seen in sperm viability (%) in the ACV group as compared with the control. When compared to the control, a prominent increase in percentage of sperm viability ($p<0.05$) in the ACV group was noticed. A significant decreased in sperm viability (%) was observed in Furan+ ACV groups ($p<0.05$) in comparison with the control group as well as the ACV group (Table 3).

Daily Sperm Production:

In comparison to control group, daily sperm production is remarkable reduce in rats treated with furan ($p<0.001$) was observed. There was a non-significant increase observed in daily sperm production among ACV ($p=0.56$) and Furan +ACV ($p=0.19$) groups in comparison with control group. In ACV and Furan + ACV treated groups ($p<0.01$) a significant rise in daily sperm production were noticed in comparison with furan group. While non significantly decrease ($p=0.004$) was evident in daily sperm production in Furan +ACV groups in comparison with furan group (Table 3).

Table 3: Mean ± SEM sperm parameters among adult male Sprague Dawley rats following treatment with furan and *Adiantum capillus-veneris* L. leaf extract.

Parameters	Control	Furan	ACV	Furan + ACV	P value statistics
Sperm motility (%)	62.2±4.07	42.3±2.59^{a***}	65.30±2.59^{b**}	63.2±2.56^{b**}	0.001
Sperm viability (%)	82.2±2.46	62.2±4.07^{a***}	84.30±2.49^{b*}	83.3±2.51^{a*b*}	0.001
Daily sperm production×10^6/Testis	2.23±4.47	1.69±1.33^{a***}	2.26±8.54 ^{b**}	2.25±1.31 ^{b**}	0.001

*p< 0.05, **p< 0.01, ***p< 0.001, values are stated as mean ± SEM showing significant variance respectively.

a value used in the comparison of control, while b value used in the comparison of furan and c value used in the comparison of ACV

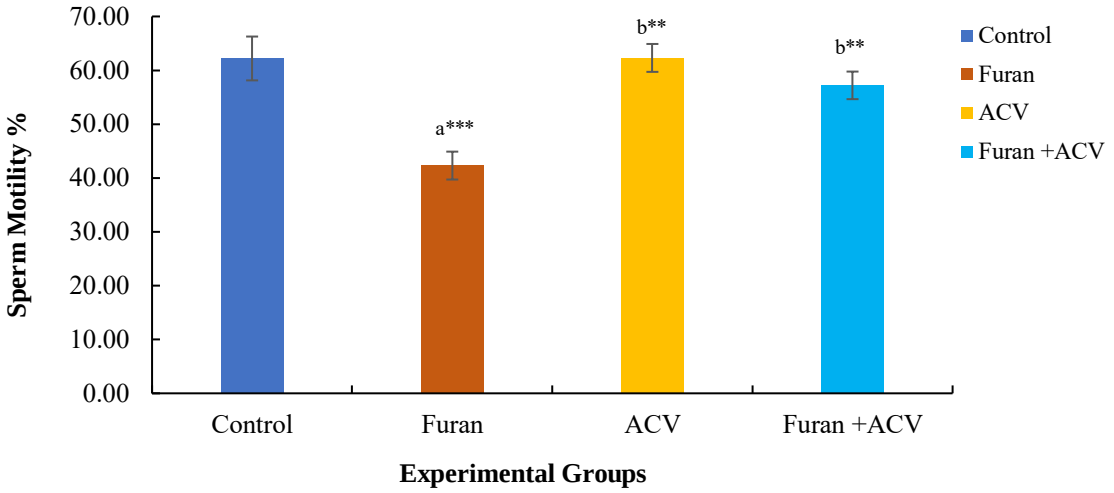

Figure 05: Mean ± SEM % rate of sperm motility among adult male rats following treatment to furan and *Adiantum capillus-veneris* L. leaf extract.

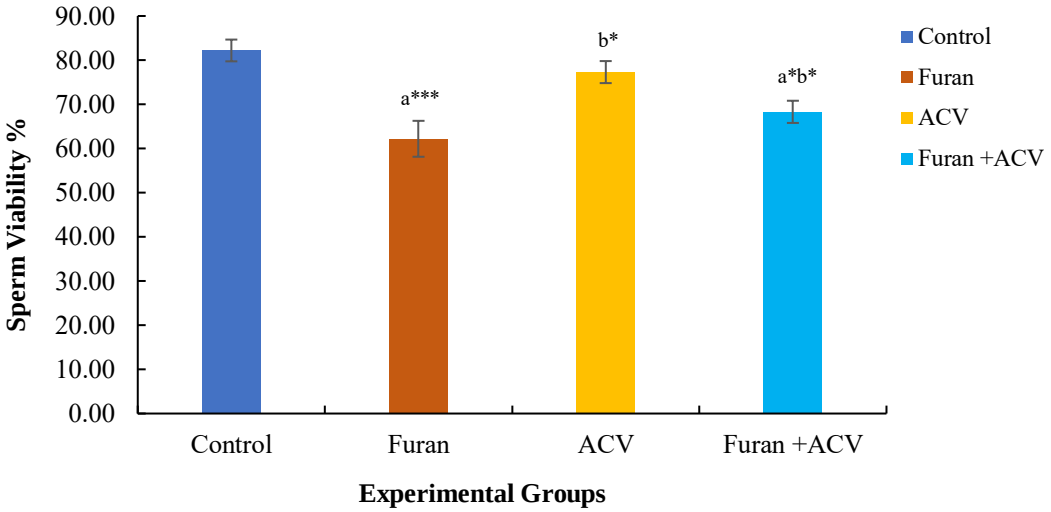

Figure 06: Mean ± SEM % rate of sperm viability rate among adult male rats following treatment to furan and *Adiantum capillus-veneris* L. leaf extract.

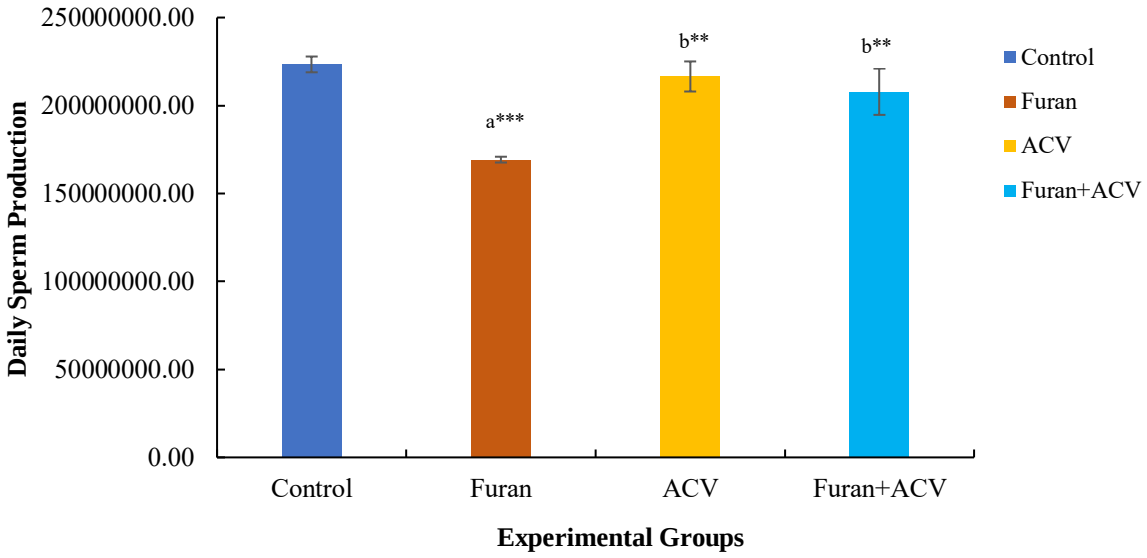

Figure 07: Mean ± SEM daily sperm production among adult male rats following treatment to furan and *Adiantum capillus-veneris* L. leaf extract.

Lipid Profile:

Cholesterol:

Significant elevations were observed in the cholesterol levels among the furan-treated group, ACV group ($p<0.001$), and Furan + ACV treatment group ($p<0.001$) when compared with the control group. When compared with furan group, a highly significant decline in the levels of cholesterol among the ACV and Furan + ACV treatment groups ($p<0.001$) was noticed. When a comparison was made with the ACV group, a prominent elevation in the levels of cholesterol in Furan + ACV group ($p<0.001$) was noticed (Table 4).

Triglycerides:

A significant increase was seen in triglycerides level in the furan treated group ($P<0.05$) when compared with the control group. While a significant decrease in the level of triglycerides was evident in the ACV group ($P<0.05$) when compared with the furan group. However, there was no significant change among the ACV ($p=0.80$) and Furan + ACV ($p=0.60$) groups in comparison with the control and furan groups (Table 4).

High-density lipoprotein (HDL):

A remarkable decrease ($P<0.001$) in HDL levels was evident in the furan group in comparison with control. There was a non- significant increase ($p=0.09$) among ACV and Furan + ACV treated groups in comparison with the control. While, in ACV group and Furan + ACV group ($P<0.001$), a significant rise in the levels of HDL was evident when compared with the furan group. Non-significantly increase ($p=0.19$) was evident in Furan + ACV treatment groups when comparison made with the ACV group (Table 4).

Low-density lipoprotein (LDL):

In furan treated group, a remarkable increase ($p<0.001$) was detected in levels of LDL in comparison with control group. While in the ACV group, a non-significant decrease ($p=0.10$) was experienced in comparison with the control group. A significant decrease ($p<0.001$) was observed in LDL levels of Furan + ACV group when a comparison was made with the control group. A prominent decrease ($p<0.001$) in the levels of LDL was observed in ACV as compared to the furan group. However, cortisol levels in the Furan+ ACV group were found to be decreased non-significantly ($p=0.19$) than in furan group.

When compared to ACV group, significant elevation in levels of LDL in the Furan + ACV treated group ($p<0.001$) was noticed (Table 4).

Table: 4 Mean ± SEM Cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein concentration (mg/dL) among adult male Sprague Dawley rats following treatment with furan and *Adiantum capillus-veneris* L. leaf extract.

Parameters	Control	Furan	ACV	Furan + ACV	p value statistics
Cholesterol (mg/dL)	143.56±3.63	286.16±3.93^{a***}	184.93±4.44^{ab***}	236.30±5.90^{abc***}	0.001
Triglycerides (mg/dL)	100.43±1.28	159.13±14.9^{a*}	106.09±2.02 ^{b*}	116.00±4.81	0.05
HDL (mg/dL)	60.59±2.25	38.56±3.43^{a***}	62.43±3.43 ^{b***}	67.93±2.25 ^{b***}	0.001
LDL (mg/dL)	48.05±0.72	93.35±2.12^{a***}	41.66±1.61 ^{b**}	46.85±1.06 ^{ac**}	0.001

*p< 0.05, **p< 0.01, ***p< 0.001 these values are stated as mean ± SEM showing significant variance respectively.

a value used in the comparison of control, while b value used in the comparison of furan and c value used in the comparison of ACV

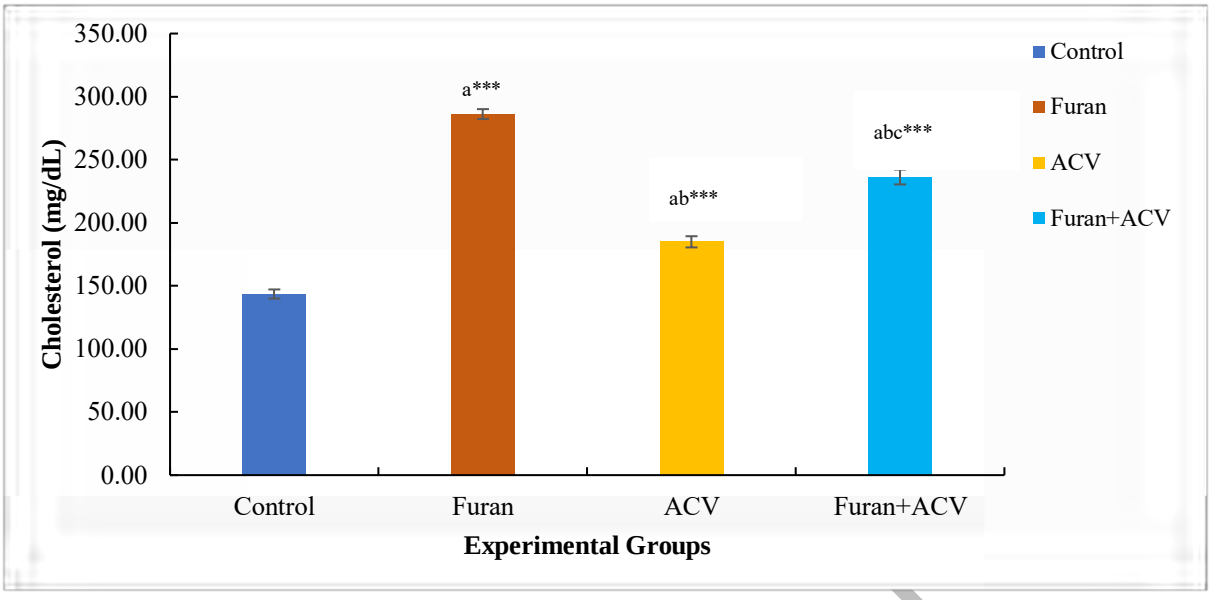

Figure 08: Mean ± SEM cholesterol levels (mg/dL) among adult male Sprague Dawley rats following treatment with furan and *Adiantum capillus-veneris* L. leaf extract.

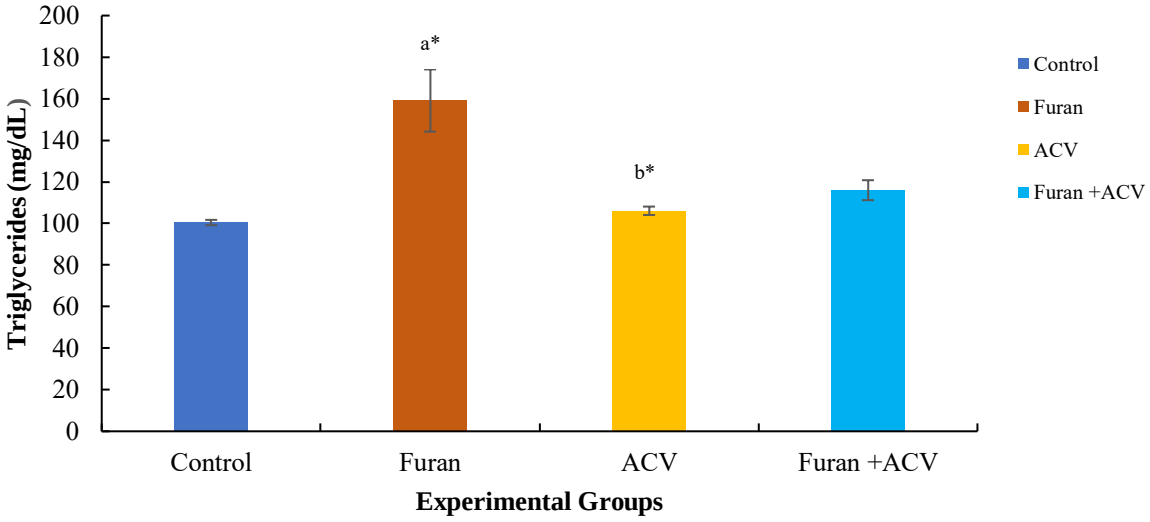

Figure 09: Mean ± SEM triglycerides levels (mg/dL) among adult male Sprague Dawley rats following treatment with furan and *Adiantum capillus-veneris* L. leaf extract.

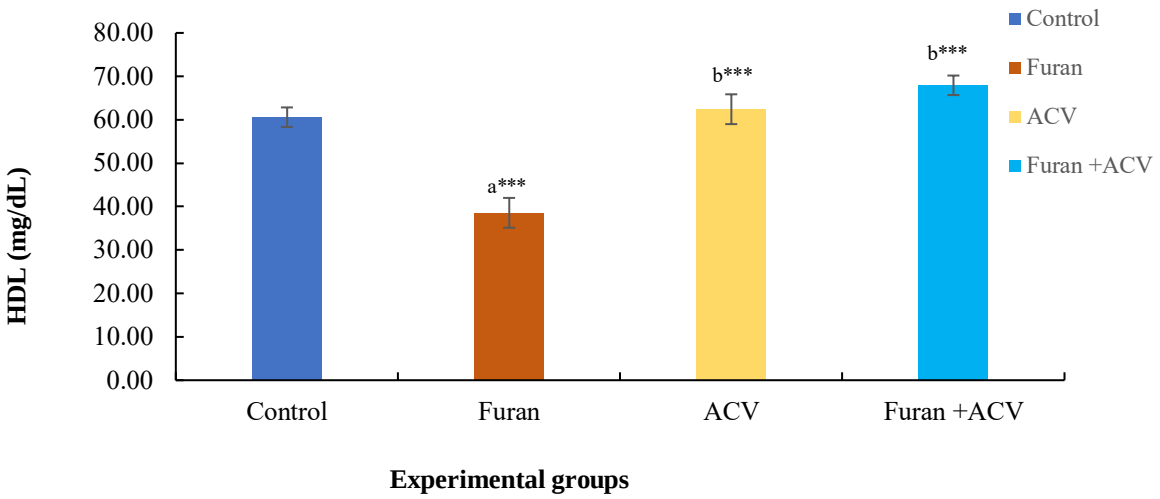

Figure 10: Mean ± SEM HDL levels (mg/dL) among adult male Sprague Dawley rats following treatment with furan and *Adiantum capillus-veneris* L. leaf extract.

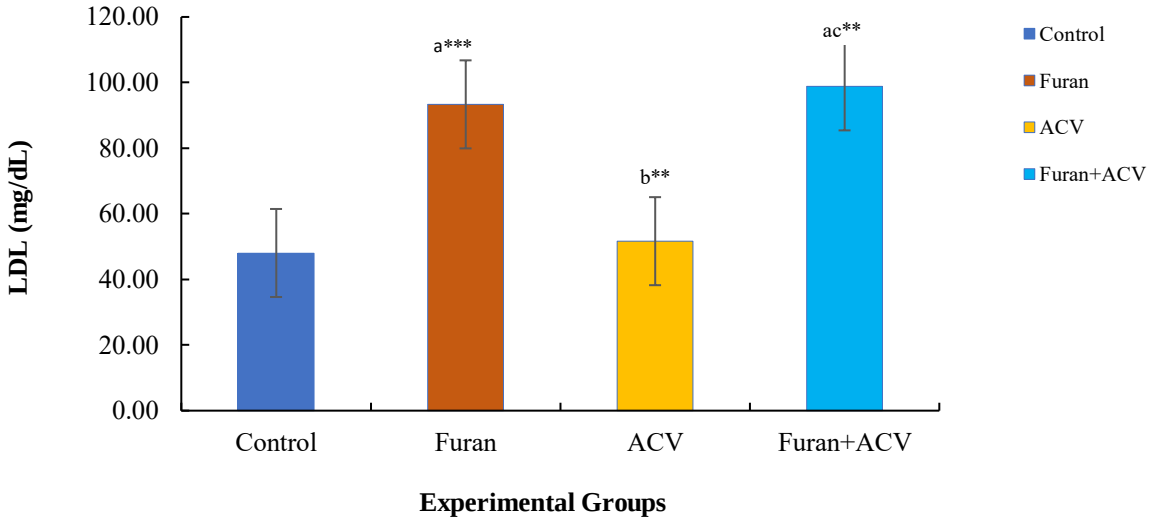

Figure 11: Mean ± SEM LDL levels (mg/dL) among adult male Sprague Dawley rats following treatment with furan and *Adiantum capillus-veneris* L. leaf extract.

Hormonal Analysis:

The concentration of testosterone significantly reduced in rats treated with furan ($p<0.001$) and non-significant increase ($p=0.49$) in levels of testosterone was evident among ACV and Furan+ ACV groups when comparison was made with the control group. When compared with the furan group, a highly significant increase ($p<0.001$) in levels of testosterone among ACV and Furan+ ACV treatment groups was observed. However, a non-significant rise ($p=0.17$) was seen in levels of testosterone in the Furan+ ACV treated group in comparison with ACV group (Table 5).

A remarkable increase in levels of cortisol was detected in furan treated rats ($p<0.001$) in comparison with the control. There was non-significant rise ($p=0.11$) in concentration of cortisol in ACV in comparison with control group While, significant increase was observed in cortisol levels in Furan+ ACV group ($p<0.05$) as compared to the control group. Cortisol levels in ACV group were significantly decreased ($p<0.05$) when compared with the furan group. However, when compared to the furan group, levels of cortisol were found to be non-significant decreased ($p=0.80$) in Furan+ ACV group. When comparing the Furan+ ACV groups to the ACV group, there was a non-significant increase in cortisol levels ($p=0.50$) (Table 5).

Table 5: Mean ± SEM plasma levels of testosterone and cortisol (ng/mL) among adult male Sprague Dawley rats following treatment with furan and *Adiantum capillus-veneris* L. leaf extract.

Parameter	Control	Furan	ACV	Furan + ACV	P value statistics
Testosterone (ng/mL)	4.16±0.29	2.66±0.22 ^{a***}	4.46±0.32^{b***}	4.66±0.31^{b***}	0.001
Cortisol (ng/mL)	75.4±4.41	112.1±7.27^{a***}	90.1±4.42^{b*}	96.08±7.44^{a*}	0.006

*p< 0.05, **p< 0.01, ***p< 0.001 these Values are stated as mean ± SEM showing significant variance respectively.

a value used in the comparison of control, while b value used in the comparison of furan and c value used in the comparison of ACV

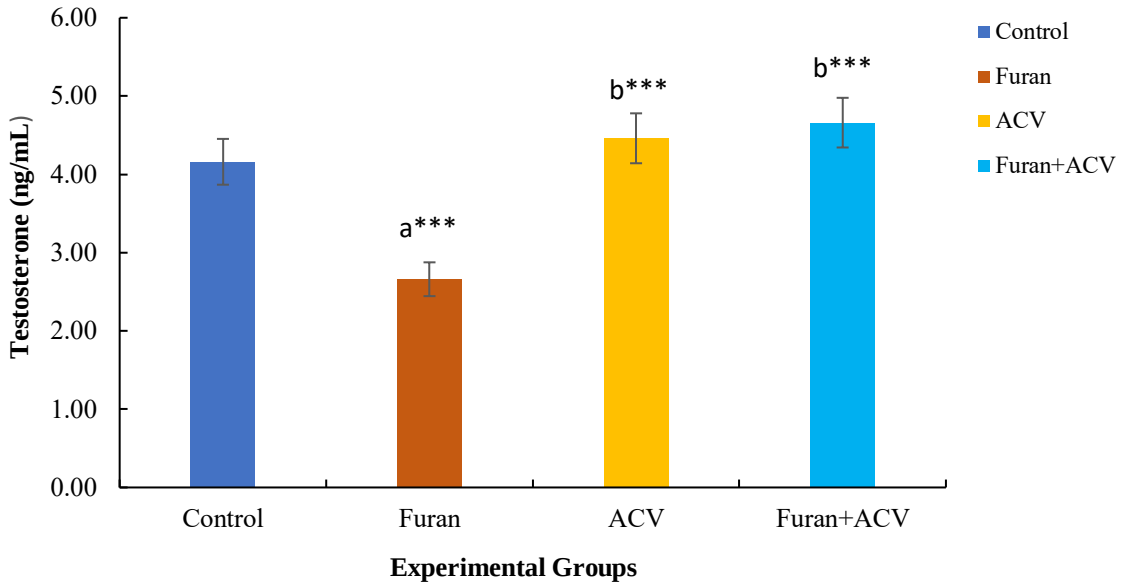

Figure 12: Mean ± SEM Testosterone levels (ng/mL) among adult male Sprague Dawley rats following treatment with furan and *Adiantum capillus-veneris* L. leaf extract.

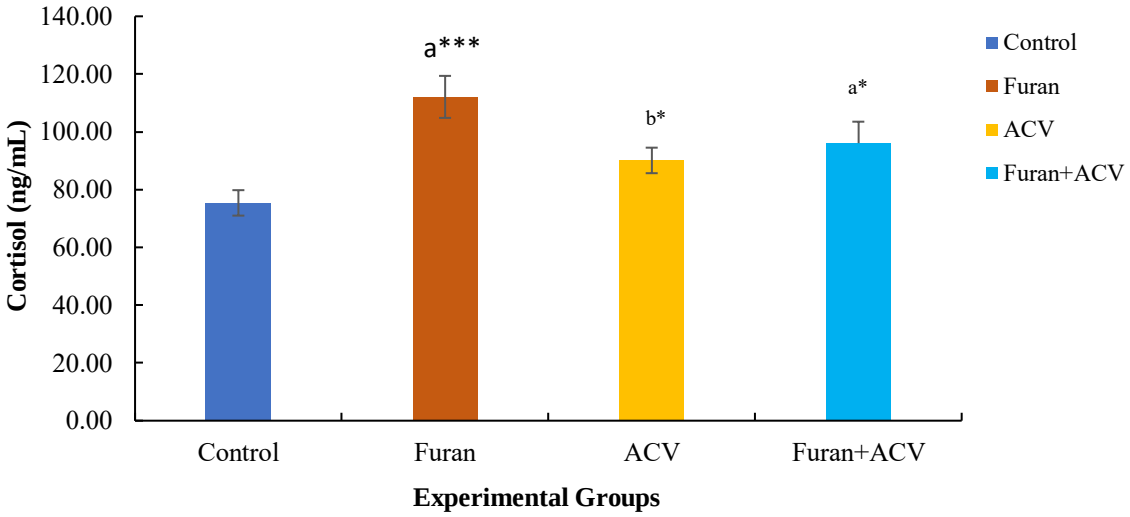

Figure 13: Mean ± SEM cortisol levels (ng/mL) among adult male Sprague Dawley rats following treatment with furan and *Adiantum capillus-veneris* L. leaf extract.

DISCUSSION

Furan is a highly volatile heterocyclic organic molecule. It has been classified as a probable human carcinogen (*IARC* 1995*;* NTP 1995). Recently, furan has been recorded in many heats treated foods, including vegetables, baby foods, sauces, canned and jarred, and soups (US FDA 2004). As a result, the presence of furan in baby foods, cereals and coffee may have a considerable impact on overall human to furan exposure, necessitating a greater understanding of the health concerns linked with their intake (Rahn & Yeretzian, 2019). It has been reported that reproductive problems by disturbing spermatogenesis caused by furan exposure, therefore, lead to cell death in germ and Leydig cells. Thus, furan is of high concern on a global scale, because of its adverse effect on mammals including humans (Hamadeh *et al*., 2004). A remarkable number of modern drugs have been developed and isolated from plant sources that are known to be effective against chemicals that act as endocrine disruptors (Mustapha, 2013). Because of the therapeutic and nutritional value of *Adiantum capillus-veneris* L., it was used as herbal medicine (Al-Snafi, 2015). Therefore, the current study aimed at determine to protective effects of *Adiantum capillus- veneris* L. against toxicity caused by furan in adult male Sprague Dawley rats. To conduct a present study, adult male rats were orally administrated with 40mg/kg of furan and 250mg/kg of methanolic leaf extract of *Adiantum capillus-veneris* L. for 28 consecutive days.

Measurements of body weight alone can be used to determine the efficacy of weight loss therapy (Blackwell, 2002). In current study, results showed body weight gain and weight of accessory organ decrease significantly in furan group in comparison to control group. Our results are in line with the previous studies conducted by Rehman *et al.* (2019), where body weight and accessory organ weight among furan administered group (40mg/kg) showed a decrease in body weight and accessory organ weight (Rehman *et al*., 2019). In rats, oral administration of furan is capable of easily passing through a biological membrane and is rapidly absorbed from the intestines, however, primary targets of toxicity of furan is liver, where P-450 cytochrome enzymes rapidly metabolizes furan to form cis-2-butene1,4-dialdehyde (BDA) and CO_2, and BDA is the main metabolite, and is considered cytotoxic and capable of irreversibly binding with nucleosides and proteins (Hamilton *et al*., 2006; Chapin *et al*., 1997). The underline pathway by which furan

impacts body weight gain is not known, however, it could be an indirect effect seen by changes in plasma hormones or direct effects on food intake (Mårin *et al*., 1992). Further, in ACV treated group (250mg/kg) body weight gain and weight of accessory organs is non-significantly increased was detected in comparison with control, as supported by previous studies by Gaikwad *et al*. (2013), where higher doses of ACV (500mg/kg) showed a remarkable increase in body weight in rats (Kanchan Gaikwad, 2013). Body weight is found to increase non-significantly in the ACV + furan treatment group in comparison to control was observed. Our results are similar to the previous findings, where decreased body weights following bisphenol A and cisplatin exposure was restored by the protective effects of *Adiantum capillus-veneris* L. in rats (Kanchan Gaikwad, 2013; Yousaf *et al*., 2016).

For normal fertilization sperm motility is necessary and for the evaluation of ejaculated sperm's fertilizing capacity, sperm motility is considered the most important factor. In the present study, viability, and motility of sperm and DSP rate is found to decrease significantly was noticed in the furan treated group (40mg/kg) in comparison to the control group. A similar result was also previously published by Rehman *et al*. (2019), where a decrease in sperm viability and motility after furan exposure was noticed. Similar observations were reported by Uzunhisarcikli *et al*. (2007), where a reduction in sperm count was caused as a result of a decrease in levels of testosterone. Recent literature also reported that spermatozoa exposed to high levels of reactive oxygen species result in decreased viability and motility of sperm, which leads to infertility, however cell-to-cell interaction and spermatogenesis and within the testis controlled by testosterone (Aitken *et al*., 2011). The result of the present study could be explained by the lower levels of testosterone. As the normal concentration of testosterone is required for the normal structure maintenance and accessory sex organs functions, a decreased in the rate of DSP can be caused by decreased levels of testosterone in the blood (Sethi *et al*., 2010). The increased levels of cortisol might lead to suppression of spermatogenesis and cause disruption in spermiation, and impairment of sperm quality (Pressman *et al*., 2018; Castranova *et al*., 2005). Longer exposure to furan was linked to cell death in Leydig cells and germ cells and as well as lower LH and testosterone levels (Karacaoğlu *et al*., 2010). Further, non-significant increase in sperm viability, motility and daily sperm production in ACV (250mg/kg) treated group was observed as reported by Yousaf *et al*. (2016) have an

androgenic effects by increasing sperm viability, motility, and daily sperm production. Sperm viability, motility, and daily sperm production also non significantly increased in Furan + ACV groups as compared to control group. As described previously by Yousaf *et al*. (2016), the decreased sperm viability, motility and daily sperm production following bisphenol A exposure was restored by the protective effect of plant *Adiantum capillus-veneris* L. in rats.

A lipid profile help in determining the concentration of lipids, such as cholesterol and triglycerides present in the bloodstream. (Ngala *et al*., 2018). In different body organs and cells, lipids perform vital functional and structural roles as well as maintain the normal body processes (Rawi *et al*., 2012). The present study indicated a highly significant increase in plasma cholesterol, LDL, and triglycerides levels, while HDL was decreased in furan group (40mg/kg) in comparison to the control. These findings are in line with the earlier studies, conducted by Rehman *et al*. (2019), where after furan exposure, levels of plasma triglyceride, total cholesterol, and LDL were elevated, while the levels of HDL decreased. Previous literature also reported that oxidative stress induces aberrant serum lipid concentrations (Martins *et al*., 2018). Increased fatty acids production causes a decrease plasma HDL level, and a rise of plasma cholesterol concentration, indicatory to liver dysfunction. Because normal levels of HDL and LDL are indicators of proper functioning of the liver. In a clinical test, a ratio of TG/HDL levels is used for detecting people who appear to be healthy individuals but have cardiovascular and metabolic impairments (Raju *et al*., 2011; Ghanayem *et al*., 2010). Lowered HDL levels can be explained by the fact that during early phases of development, plasma lipoproteins levels are low (Brai *et al*., 2020). Another description for the detected change in levels of plasma HDL and LDL is that the release of hepatic triglycerides into the blood is inhibited by different liver toxins (Koszucka *et al*., 2020). For example, acrylamide, cause changes in the same way (Ghanayem *et al*., 2010.; Raju *et al*., 2011). It can explain that observed low triglycerides levels that may lead to an accumulation of triglycerides in the liver, which caused fibrosis and liver damage in overweight rats (Rehman*et al*., 2019). The result of the present study might be similar to the observation stated by Ldeniz *et al*. (2011) that LDL receptors overactivation might be caused due to increased levels of plasma LDL. In later findings, that furan exposure in male rats had elevated LDL levels in blood plasma and LDL receptors assist the entry of cholesterol molecules in the normal human body cells.

The low-density lipoproteins release their cholesterol and triglycerides on attachment to their receptors on the hepatocytes (Shah *et al*., 2022). Because of elevated levels of cholesterol, caused by decreased low-density lipoproteins transport into cells, the formation of new LDL receptors was ceased. Limiting the LDL intake and the non-functional receptors improves the serum cholesterol levels (Aldred, 2008). Increased free cholesterol levels prevent the synthesis of the receptor of LDL, thus more accumulation of cholesterol is promoted by less intake of LDL (M, 2001). Previously, researchers have also reported that low levels of testosterone, abnormalities of sperm, and male infertility might be linked to increased cholesterol and reduced HDL levels (Shalaby *et al*., 2004). However, the result of the present study in ACV treated group (250 mg/kg) decreased levels of plasma cholesterol levels, LDL, and triglycerides, while an increase in levels of HDL was observed in comparison to the control group; these findings are similar with previous studies, where high cholesterol diet-fed rats exposed to *Adiantum capillus-veneris* L. results showed the total cholesterol (TC), LDL, and triglycerides levels were all reduced but did not affect HDL levels, because *Adiantum capillus-veneris* L. exhibited hypocholesterolemic property (Al-Hallaq *et al*., 2015). Plasma cholesterol LDL and triglycerides levels were also decreased while HDL levels were also increased in the ACV+Furan treatment group, these findings are following previous studies conducted by Kanchan Gaikwad, (2013); Yousaf *et al*. (2016) significantly decreased serum LDL levels and increased HDL level following bisphenol A and cisplatin exposure was restored by protective effects of *Adiantum capillus-veneris* L. in rats.

The maturation and development of spermatozoa and normal steroidogenesis are important for male infertility. Normal production and release of gonadotrophin (LH, FSH) are controlled by the phenomena of spermatogenesis and steroidogenesis in testes (Rehman *et al*., 2019) The normal secretion of testosterone is under the control of luteinizing hormone The appropriate concentration of testosterone is important for the normal function of the testes. In the current study, the hormonal concentration of plasma testosterone was significantly reduced when rats were treated with furan (40mg/kg) in comparison to the control. Formerly, similar results have been reported that reduced testosterone levels after exposure to the higher dose of furan (Rehman *et al*., 2019). However, reduced testosterone levels are a sign of biochemical toxicity (Yoshida *et al*., 2002). Recently, literature reported that oxidative stress might be caused decreased levels

of testosterone and increased spermatogenesis sensitivity, which is closely associated with primary functions of antioxidant enzymes in Leydig cells (Cao *et al*., 2008; Rezvanfar *et al*., 2013). The present study results can be explained that normal function of Leydig cells could be affected after furan exposure, result decreased testosterone levels. The reduction in T concentration is significantly contributed by the reduction in luteinizing hormone in male rats which encourages spermatogenic arrest and infertility. Non -significant increased testosterone concentration in ACV treated group (250mg/kg) was observed when a comparison was made with the control group. The result of present study was similar to previous study shown by Tawab *et al*. (2014), which stated that for the treatment of uncontrolled ejaculation, *Adiantum capillus-veneris* L. is used as an indigenous or folk medicine. Moreover, its ability to increase testosterone levels in blood prove its protective effects on the male reproductive system. Testosterone concentrations were increased in Furan + ACV treated group in comparison with the control group. Similar findings were detected by Yousaf *et al*. (2016), where following exposure to bisphenol A, testosterone concentration was restored by protective effects of *Adiantum capillus-veneris* L. in rats.

In the current study, increase cortisol levels were detected in the animals of furan treated group (40mg/kg) when a comparison was made with the control group. These findings are in accordance with previous research(Rehman *et al*., 2019). The increased cortisol levels have been linked with a reduced synthesis of growth hormones and sex steroids in multiple findings (Liening *et al*., 2010; L. J. Chen *et al*., 1997). PCBs, as well as heavy metals are endocrine disruptors that might be cause an increase levels of cortisol in mammals and fish (Tort *et al*., 2011; Sumera *et al*., 2018; Tan *et al*., 2007). Elevated cortisol causes energetic costs of reproduction (Katrine Knutsen *et al*., 2017; Leal *et al*., 2011). Previous findings elaborate higher concentration that could be used to determine abnormal development of reproductive organs caused by low level of testosterone. No significant rise in cortisol levels was detected in ACV treated group (250mg/kg) in comparison with the control group. No previous study is available to describe the effect of *Adiantum capillus-veneris* L. on cortisol levels. A significant increase in cortisol levels was seen in the Furan+ACV treatment group as compared to the control group. Literature regarding the protective effect of Adiantum *capillus-veneris* L. on cortisol secretion is still lacking.

Conclusion

The results of the present study showed that a high concentration of furan can have antiandrogenic effects on the genital system in adult male rats resulting in decrease a body weight gain, lowered sperm motility, viability, and daily sperm production rate, increase in cholesterol, triglyceride, low-density lipoproteins (LDL) and decrease in high-density lipoproteins (HDL), as well as a reduction in testosterone and increase in cortisol levels, *Adiantum capillus-veneris* L help amerolatively the effect of furan toxicity. To understand the underlying mechanism of action of furan in reproduction at a cellular and molecular levels, need further studied.

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modern drugs have been developed and isolated from plant sources that are known to be effective against chemicals that act as endocrine disruptors. Because of the therapeutic and nutritional value of Adiantum

capillus-veneris L., it has been used as herbal medicine.

17**Therefore, the** goal **of the current study was to** determine ameliorative **effects of**

Adiantum capillus- veneris L. against furan-induced

52**toxicity in adult Sprague Dawley** male **rats**

. To conduct a present study,

69**adult male Sprague Dawley rats were separated into 4 groups**

(n=5); Group I was given orally with 0.9% normal saline, while Group II was orally administrated with 40mg/kg of furan, Group 3 was orally administered with 250mg/kg methanolic leaf extract of Adiantum capillus-veneris L., and Group 4 was orally administrated with furan and ACV for28 consecutive days, respectively. On the 29th day, animals were weighed, and decapitated; and samples of tissue (testis and epididymis) and blood were collected for sperm parameters and biochemical analysis. Blood was subjected to centrifugation, followed by plasma collection, and was later, stored at -20˚C for biochemical and hormonal analysis. The present study results showed a remarkable decrease in daily sperm production rate and percentage of viability and motility of sperm in furan group in comparison to control group. Analysis of lipid profile indicated an increase in

51**levels of cholesterol, low- density lipoprotein** (LDL), **and triglycerides, and** a decrease in **levels of high-density lipoprotein** (HDL) **in**

the furan administered group. The findings of hormonal analysis depicted a reduction in testosterone levels, while increased cortisol concentrations were evident in furan- treated groups. For all the studied parameters, Adiantum capillus-veneris L. showed an opposing result than that of furan treated group. Conclusively, plant Adiantum capillus-veneris L. overwhelm androgenic effect of furan on rat's reproductive system and protect the rats' testes against toxicity caused by furan that appears to be conciliated. Therefore, it is suggested that due to the protective effect of plant Adiantum capillus-veneris L. in male rats, it can be used to restore the toxic actions mediated by endocrine disruptors. Introduction Recently, the focus of scientific literature has been increased on endocrine-disrupting chemicals (EDCs) and their importance in human pathophysiology. EDCs are characterized as "exogenous chemicals substance that disturbs hormonal activities or causes disruption in the hormonal pathway (Gore et al., 2015; Thomas Zoeller et al., 2012). Three types of actions have been shown by EDCs (1) endocrine action; (2) pathologic endocrine mediated- action; (3) affect the association between substance and endocrine action (Alexander et al., 2013; Slama et al., 2016). EDCs are the artificial exogenous chemical that regulates genomic expression, and also interfere with the endocrine system proposes by the European Food Safety Authority (EFSA) (Zama & Uzumcu, 2010). EDCs have been grouped into five main categories: industrial includes polychlorinated dioxins, polychlorinated biphenyls (PCBs), and alkylphenol, agricultural such as insecticides, weed killer, insect killer, antifungal agents, households such as phthalates, polybrominated biphen, bisphenol A, and drugs and heavy metals that include lead, cadmium, mercury, and arsenic and food-based acrylamide and furan (De Coster & Van Larebeke, 2012; Kabir et al., 2015; Monneret, 2017). EDCs have contaminated the human body through various pathways. Common exposure pathways such as inhalation, ingestion, and direct contact via skin. EDCs move into the food chain and accumulate in animals and humans body by using these various pathways (Balaguer et al., 2017; Dickerson & Gore, 2007; Kabir et al., 2015b). EDCs are highly lipophilic

and possess a long half-life, hence, accumulated in the adipose tissues (Disruptors & 2015, 2015; Schug et al., 2011; Thomas Zoeller et al., 2012). EDCs bind and activate different hormone receptors such as glucocorticoid receptor (GR),

> 65**androgen receptor (AR**), thyroid hormone **receptor** (TR), **estrogen-related receptor (ERR), aryl hydrocarbon receptor (AhR**

), estrogen receptor (ER), retinoid X receptor, and also mimic actions of natural hormones (Monneret, 2017; Balaguer

> 50**et al., 2017**; Heindel **et al., 2015**; Mnif **et al**

., 2011). EDCs may also impair the production, transportation, metabolism, and removal of hormones, lowering concentrations of endogenous hormones (Mnif et al., 2011; Thomas Zoeller et al., 2012). EDCs have additive or synergistic effects on the endocrine system (Barouki, 2017; Nohynek et al., 2013). The actions of EDCs are not confined to particular axis or organ, the different axis of the body is the main target of EDCs including

> 59**the hypothalamus-pituitary-adrenal axis, hypothalamus-pituitary-thyroid axis, hypothalamus-pituitary-gonadal axis**

(Thomas Zoeller et al., 2012). Evidence shows that the central nervous system (CNS) may also be affected by EDCs, mediated by improper functioning of the hypothalamic and pituitary gland, which may affect normal activities of peripheral glands with unknown effects (De Coster & Van Larebeke, 2012; Kabir et al., 2015a). EDCs may altering adipose tissue, disrupting

> 46**endocrine regulation of adipose tissue and** synthesis of **adipocytokine**, reduced **basal metabolic rate, and** modifying **the** hunger **and satiety** control (**Street** et al., **2018**

). EDCs can affect behavior in both males and females in different mammals including humans. Even at very low doses, through epigenetic changes EDCs changing the normal steroidogenesis pathway in males, also affect the development of rodent brain (Masuo & Ishido, 2011). The EDCs can mimic sex gonadal hormones and can bind to ERs , which can interfere with the signaling of hormonal because the reproduction is affected by EDC's actions (Monneret, 2017). The risk of breast cancer may increase following exposure to, polychlorinated biphenyls (PCBs), polychlorinated dioxins, cadmium, furans, and ethylene oxide. Heavy metals and pesticide exposure appear to increase the risk of prostate carcinogenesis (De Coster & Van Larebeke, 2012). Recent scientific literature showed that EDCs exposure during the development period not only damages the living organisms but also affects future generations. (Lauretta et al., 2019). Heat-induced food toxicants: Thermal processing is used to preserve food,

> 5**especially in the** formation **of shelf-stable foods with** certain **nutritional**

qualities.

5**On the other hand, it** results in **the formation of heat- induced toxic compounds**, known as **thermal process**

pollutants, which have toxicological effects, and pose

5**health risks to humans. Acrylamide, chloropropanols, and furan are well-known as thermal process** chemicals **in foods**

(Maga & Katz, 2009; Tareke et al., 2002). Chloropropanols and furan were discovered in food in the 1970s, while acrylamide was discovered in 2002 (Mogol & Gö, 2016).

11**The International Agency for Research on Cancer has classified furan as a** probable **human carcinogen (IARC** 1995). **Furan**

: Furan (C4H4O) is a highly volatile organic heterocyclic molecule. Furan has been classified as a probable

49**human carcinogen by the National Toxicology Program (NTP**) and US **Department of Health and Human Services and the**

76**international agency for research on cancer (IARC) (IARC**

1995; NTP 1995). In year-long gavage research, even at low doses, furan was found to be induced cancer in various experimental animal species including rodents (Batool et al., 2021). Furan and furan-contained chemicals was first detected in meals, during research presence of furan aimed at detecting volatile molecules created during heating, which are responsible for the odour of processed foods. In 1983, presence of furan in volatile components of coffee was seen and after few years it was confirmed (Merritt et al., 2002). Previous literature reported that the chemical was present in various foodstuffs such as chicken, bread, and tinned beef. Initially, carbohydrate that is thermally breakdown is closely linked with furan formation (Maga & Katz, 2009). Recently, furan has been discovered in many heats treated foods, including vegetables, baby foods, sauces, canned and jarred, and soups (US FDA 2004). Carbohydrates and amino acids are present in coffee, which break down at higher roasting temperatures and react with one another by different mechanisms and following in the generation of furan to greater extents (Rahn & Yeretzian, 2019). Recent literature reported the occurrence of furan in coffees that are available easily in markets (Becalski et al., 2016; Chaichi et al., 2014). As a result, the existence of furan in various items such as baby foods cereals and coffee may

48**have a** considerable **impact on overall human to** furan exposure, necessitating **a**

greater understanding of the health concerns linked with their intake (Rahn & Yeretzian, 2019). Figure 01: Chemical structure of Furan (Rahn & Yeretzian, 2019) Furan Formation: Baking, roasting, pasteurizing, cooking, and sterilization are thermally driven processes that involve furan formation (C. Crews et al., 2007). Because of its carcinogenicity and presence in many heat treated foods, such as meat products, coffees, and cereal, has gotten a lot of attention around the world (Altaki et al., 2017; EFSA, 2010). Absorption of

furan through the gastrointestinal tract and furan is the breakdown by P450 2E1 hepatic cytochrome mediated ring-opening, which leads to the

> 90**formation of cis-2-butene-1,4**

-diol, which is

> 31**a highly reactive metabolite that can react with** amino **and** thiol **groups in glutathione and other peptides (Chen et al**., 1995; Lu **et al**

., 2010),.

> 27**Nevertheless, their mechanisms of formation remain unclear**

. The

> 3**thermal degradation of carbohydrates and ascorbic acid and its derivatives and** as well as **thermal oxidation of polyunsaturated fatty acids**

have been considered as potential precursors and alternate pathways for the production of furan in meals, rather than a single mechanism (C. Crews et al.,

> 3**2007). Perez-Locas and Yaylayan (2004) suggested that ascorbic acid** has **the** greatest **potential** for producing **furan, followed by some sugar/amino acids mixtures; reaction conditions, such as temperature, time, and pH, can also**

have a significant impact on

> 27**furan formation (Fan, Huang & Sokorai, 2008**

). Figure 02: Synthesis of furan from various

> 27**precursors such as carbohydrates, amino acids, and**

polyunsaturated fatty acids through different pathways. Exposure to furan: Human food contains thousands of physically different chemical substances, the majority of which are of natural origin and components that are added such as colorants, nutrients, and flavors. Furthermore, maximum furan contents were discovered with heat treatment of baby food, meat products, cereals products, soups, and sauces (EFSA, 2010). Furan levels were found to be higher in nutritious drinks and bakery products (Katrine Knutsen et al., 2017; FDA, 2004). The amount of furan content in various food substances was used to determine human exposure to furan. Adult humans in developed countries are exposed to furan at a rate of 0.34 to 1.23

> 71**g/kg BW/day** around **0.78 g/kg BW/day**

(EFSA, 2010). The average daily exposure at the age of 3-12 months is 0.27 to

21**1.01 g/kg BW/day**

(Altaki et al., 2017). 0.

21**23- 1.77 g/kg BW/day of** furan level **was** reported in **the**

newborn (Minorczyk et al., 2011). Coffee is the most abundant source of exposure in adults (Altaki et al., 2017). Breastfeeding or formula feeding is the main source of toxicants furan in infants (Lehmann et al., 2018). High levels of furan were observed in

21**baby carrot juices, prune juices, and flavors of**

nutritional drinks (J.-W. Wegener & López-Sánchez, 2010). Recent research showed that bottled and canned drinks are a significant source of furan for newborns (J. W. Wegener & López-Sánchez, 2010). Figure 03: Different routes of furan exposure Toxicology of furan Recent observations showed that furan induces several types of cancer and the humans and different animals biological system are also affected by furan exposure ((IARC 1995; Uçar & Pandir, 2017 ;Hamadeh et al., 2004). Furan has been shown to have negative effects on rodent's vital organs (Ldeniz et al., 2011; Webster et al.,2014). An elevation of serum alanine aminotransferase (ALT) is closely linked to hepatic necrosis that is caused by furan exposure in rats (Baş et al., 2016; Gill et al., 2010). In rats, exposure to furan increases levels of malondialdehyde by reducing

67**levels of glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase**

(CAT) (Baş et al., 2016). Furan altered the histology, ovarian cell's DNA structure, levels of malondialdehyde, and activities of antioxidant enzymes (Uçar & Pandir, 2017). Furan also caused DNA damage by generating reactive oxygen species in lymphocytes and sperm (Pandır, 2015). Some studies showed that furan has antiandrogenic action (Cooke et al., 2010; Karacaoğlu et al.,2011). Recent clinical literature showed that the mother's breastmilk contained a higher level of furan and dioxin than 11- week-old babies (Pluim et al., 1993). Previously, reported that furan exposure via breastfeeding or food has been linked to fetal abnormalities due to its ability to pass through the placental barrier (Krowke et al., 1990). Decreased in levels of testosterone and decreased in viability and motility of sperm and cell death in germ and Leydig cells after exposure to furan in adult male rats was stated by Cooke et al, (2014); Karacaoğlu et al, (2010). Oxidative stress caused by furan: When antioxidant levels are reduced, excessive

11**generation of reactive oxygen species** is harmful (Embola **et al**

., 2002). Excessive ROS generation

11**induced toxicity** in **the reproductive system of**

different mammals including humans. Oxidative damage can cause abnormal sperm morphology, as well as decreased sperm motility, and egg production (Iwasaki & Gagnon, 1992; Sukcharoen

89**et al., 1996**; Mazzilli **et al**., 1994; Aitken **et al**

., 2011). ROS production increases lipid peroxidase, which changes acrosome reaction, sperm DNA damage, and mucogenic capacity (Ichikawa et al.,1999). Excessive ROS damages gonadotrophins receptors, lower cyclic AMP and inhibits steroid secretion by affecting corpus luteal (Aten et al., 1992). In the presence of unsaturated fatty acids in the membrane, spermatozoa are affected by oxidative stress (Aitken et al., 2011). Mitochondria are found in spermatozoa and provide a consistent supply of energy. Excessive ROS generation affects inner and outer mitochondrial membranes and releases cytochrome C, which triggers apoptosis (Thuillier et al., 2003). Antioxidant enzyme protects living cells by reducing ROS formation as well as detoxifying ROS by hydrogen peroxide conversion and dismutation while catalase converts hydrogen peroxide to water (Imlay et al., 1988; Wiseman & Halliwell, 1996). Contaminated elements in the human body usually target the liver because the breakdown of chemicals occurred in the liver which eventually leads to hepatocyte destruction and liver toxicity (Livingstone, 1991; Patel et al.,2012.). Furan is quickly absorbed in the body and can also easily pass through biological membranes due to its low polarity (Bakhiya & Appel, 2010; D. Crews et al., 2007). Furan exposure is increased because of presence of furan in baby products and overcooked food day by day. Previous literature also showed that the occurrence of furan in human urine morning samples in various concentrations (Sudjarwo et al., 2017). Previous research reported that the primary targets of furan toxicity are kidney and liver. As the main part of the defense mechanism of the living cell, catalase (CAT) converts ROS to hydrogen peroxide which is then converted hydrogen peroxide to water, then water protects the cells from ROS toxicity (Syed et al., 2012). Furan was found to be toxic to the hepatocytes, pancreas, and renal cells. Furan is harmful to the pancreas by exerting oxidative stress (El- Habiby et al., 2017). Epigenetic changes are linked to the effect of furan (Conti et al., 2014.) Endocrine-disrupting potentials of furan Food and drug administration (FDA) previously recognized that food-based contaminants furan and acrylamide, have the potential for endocrine disruption (Robin and Clanci,2007; FDA, 2004) The normal production of steroid hormones is linked to the development of male reproductive organs such as the prostate, Wolffian ducts, epididymis, seminal vesicles, and vas deferens but food-based contaminants interact with these hormones and have a negative impact on their appropriate organ development and functioning (Cooke et al., 2014). Food-based contaminants block the enzymes 5α-reductase and aromatase, which are responsible for converting androgens to testosterone. Food-based contaminants exposure disrupts estrogen receptors (ERα and ERβ) expression levels. ERβ receptor expression in humans is sustained through morphogenesis and cellular differentiation but declines throughout early puberty. Increased levels of plasma androgens also boost the expression of the receptor that is associated with benign prostate hyperplasia and prostate cancer. The development stages of animals can also be affected by furan exposure. When animals have exposed to food-based contaminants during the stage of organ development, everlasting changes occur (Richter et al., 2007). Furan exposure disturbs the hormonal profile of species. Furan can influence behaviors and sex-specific development by disturbing the HPG axis, which is involved in the production of GnRH that contribute to reproductive maturation, and the hypothalamic- pituitary-adrenal axis, a stress mediator (Adewale et al., 2009; Schug et al., 2011). The adrenal cortex also secretes the hormone cortisol which further activates the HPA axis (Schreier et al., 2015). It has been observed that both chemical and non-chemical stressors disrupt the HPA axis (Cory-Slechta et al., 2008; Miller et al., 2007). Previous literature reported that furan has a negative impact on hypothalamic and pituitary functions (Gore et al., 2015; Kabir et al., 2015b). Furan interacts with the HPA-axis, during steroidogenesis (Martinez-Arguelles & Papadopoulos, 2015). Mercury has been found to disrupt the function of the HPA axis (Tan et al., 2009) Cortisol that is released by the HPA and HPI axes is a stress indicator in invertebrates (Barton et al., 2002; Heisler et al., 2007). The elevated level of cortisol increased the energetic costs, which affects the process of metabolism, reproduction, neurogenesis, and immune function, hypothalamus- pituitary thyroid (HPT) and HPA axes are also involved in the regulation of reproductive functions as the HPA axis influence the performance of HPG axis during development (Bernier

63**et al., 2004**.; Dobson **et al**., 2007; Hogan **et al** 2004; Leal **et al., 2011**; Tort, 2011; Sørensen **et al**

., 2011; Schreck,2010; Fitzpatrick et al., 2012). Reproductive toxicity of furan: Contaminants, pesticides, and food additives are all known to be harmful to the reproductive system (Umar Ijaz et al., 2021). Furan is one of the contaminants that may affect the reproductive system in male rats by decreasing the levels of reproductive hormones (Cooke et al.,2014; Umar Ijaz et al., 2021). Furan has also a significant impact on the testis, epididymis, and prostate gland (Luo et al.,2005; Umar Ijaz et al., 2021). Furan toxicity in the liver: The main target of furan toxicity and carcinogenicity is the liver (Gill et al., 2010). In recent findings, different serum biochemical parameters such as lipid profile, and liver enzyme are typically used to determine liver damage before histopathology confirmation (Lopez-Lopez et al., 2021) Within the liver, bilirubin conjugated

68**aspartate alkaline phosphatase (ALP**), alanine **aminotransferase (ALT), aminotransferase (AST). Total bilirubin**

is released into the circulation after cellular damage (Smith & Walker, 2002). Another complication of furan overdose is dyslipidaemia. The lipid metabolism take place in liver because liver is primary site for it and plays a significant part in serum protein synthesis. Different diseases of the liver lower the levels of cholesterol, triglyceride, and high-density lipoprotein by limiting the liver's biosynthetic capacity and potency (Subhan et al., 2012). Regardless of the toxicity of a specific organ, changes in levels of cholesterol, triglyceride, glucose, and serum proteins appear to be indicators of wide-ranging metabolic events (Smith, 2002). The mechanisms involved in these changes in different parameters are unknown. Recent literature reported that increasing the intake of fatty acids foods as an energy source to balance the lack of carbohydrate resources may take part in lowering the levels of triglycerides. Furthermore, hepatocyte injury may increase a different amino acids levels in the liver (Ramm et al., 2016) Furan Mitigation strategies: On regular basis, humans are exposed to furan through different furan contained foods, and it is essential to

16**assess the health risks associated with** furan **exposure. The**

primary source of (40-80%) dietary furan exposure is coffee in adults (Arisseto et al., 2011). However, mitigating furan is still a difficult task and there are currently no commercially accessible solutions (Anese & Suman, 2013). The various pathways involved in furan formation and as well as the importance of techniques that used for food safety and boosting aroma and flavor, as well as intricate interaction between furan retention and food matrix in food are the primary reason for this (Arisseto et al., 2010). However, no commercial techniques are still available for furan mitigation (Anese & Suman, 2013). Adiantum capillus-veneris Linn Plants and their products have been used since ancient times for food, shelter, and to cure different ailments (D'cruz et al., 2010). Traditionally, folk medicine has been used by many people around the world (Lucinda

15**et al**., 2010). **A** remarkable **number of** modern drugs **have** also **been developed and**

isolated from plant sources (Mustapha, 2013). The earliest type of healthcare known to mankind is herbal medicine. Throughout history, herbs had been used by all societies. In the evolution of modern civilization, it was an important step. With period, each community added to its knowledge base about the medicinal power of herbs in their area. Herbal medications made up a large part of today's pharmaceuticals.

84**According to World Health Organization (WHO**), herbal medicine **is** used by **the**

world's population (80 percent) currently for some part of basic health care. Because of the therapeutic and nutritional value of Adiantum capillus-veneris L. was used as herbal medicine (Al-Snafi, A. E. 2015)

29**Adiantum, a genus of** the **Adiantaceae family**

, is found worldwide,

29**from hot tropical zones to cool temperate zones**

. Sumbul and Hansraj are the local names of

29**Adiantum capillus-veneris L. in Pakistan**

(Al-Hiyasat et al., 2002). Chemical composition of maidenhair fern has, flavonoids, steroids, tannins, terpenoids, and alkaloids (Khodaie et al., 2015). The anti-inflammatory, analgesic, wound healing, anti-fungal, anti-diarrheal, anti- bacterial, anti-diabetic, antioxidant, anti-spasmodic, diuretic, and anti-urolithiasis detoxifying agent and hypocholesterolemia properties of this plant roots, rhizomes, and the fronds (Dehdari et al., 2018).An anti-inflammatory

93**effect of** the **ethanolic** leaf **extract of** this plant **on**

the toxic substances.71.15% anti-inflammatory effects were recorded at a 100 mg/kg dose of plant extract (Madboli & Seif, 2021). Due to the versatility of actions of Adiantum capillus-veneris L. plant extract is used against the toxic effects of furan. Traditional uses: The specie with potential medicinal and nutritive value was Adiantum capillus-veneris L.

30**Adiantum species were used for cough and** cold, chest complaints, **as an expectorant, to** stimulate **lactation, to help kidney function, and as** dandruff and **an antiparasitic**

. Antidandruff, laxative, refrigerant, demulcent, depurative, weak emmenagogue, emollient, weak expectorant, sudorific, galactagogue, antitussive, astringent, stimulant, and tonic were all employed on the fresh or dried leafy fronds. Tea was made from the dried fronds of the plants for the same purposes (Ahmed et al.,2012; Al-Snafi, 2015; Johnson & Sowrby, 1899). Pharmacological effects Anti-diabetic activity The presence of flavonoids and tannins in methanolic leaf extract of Adiantum capillus- veneris L. has antidiabetic potential (Ranjan et al., 2014). The plant's ability to increase weight is due to its ability to repair hepato-renal-damaged cells (Sultan et al., 2012). Furthermore, the species was found to have antihyperglycemic properties comparable to acarbose as a reference drug (Kasabri et al., 2017). Hypocholesterolemic effect The methanolic leaf extract of Adiantum capillus-veneris L. has hypocholesterolemic potential by showing a great impact on

87**levels of low-density lipoproteins and high-density**

lipoprotein and cholesterol (Al-Hallaq et al., 2015). Anti-obesity effect In vitro model, aerial parts of Adiantum capillus-veneris L. exhibited a phospholipase inhibitory effect comparable to orlistat. Reported that the most responsible phytoconstituent is chlorogenic acid (Kasabri et al., 2017). Anti-testosterone-induced hair loss effect In a testosterone-induced alopecia model in mice, the effect of methanolic leaf extract of Maidenhair fern on hair growth was evaluated and resulted increase in follicular density (Noubarani et al.,2017). Treatment of COVID-19. Ancient medics employed plant extract that might be used for treating different diseases. In modern medicine, Adiantum capillus-veneris L. also possesses pharmacological efficacy to treat many symptoms same to that caused by COVID-19 and underlying medical conditions (Hendawy, 2020). Anti-fungal and anti-microbial activity Adiantum capillus-veneris L. also contained phytoconstituents which

22**are responsible for antifungal activity against fungi (Aspergillus Niger, Aspergillus** terries, **Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Saccharomyces cerevisiae**, Fusarium sp., **etc.). These** phytoconstituents **also have antimicrobial activities**

(Rajurkar et al., 2012). Antioxidant property Adiantum capillus-veneris L. exhibits antioxidant properties because of flavonoids, reducing sugar, tannins and saponins, are present in leaves of Adiantum capillus-veneris L. (Rajurkar et al., 2012). Part used: Adiantum capillus-veneris L. with

60**fresh or dried leafy fronds**, a **dried herb with rhizome and roots** are being **used medicinally**

(Johnson & Sowrby, 1899). Distribution in the world: Adiantum capillus-veneris L. is found in places such as shady and moist all over world. It is found in Europe along the Atlantic coast as far as Ireland (Ahmed et al., 2012). Distribution in Pakistan Balakot, Changa Gali, Abbottabad (altitude 2,350 m) Mansehra, Battagram (altitude 1,100 m), Murree, Rawalpindi (altitude 1,800 m), and District Shangla are among the places where Adiantum capillus-veneris L. plant is found in Pakistan. Topography: On the globe, the Shangla district is located from 34° 31’ to 33° 08’ N and 72° 33’ to 73° 01’ E. The districts Bata gram and Kala Dhaka Springier are located on the east of district Shangla, district Swat is located on the west, district Buner is located on the south, and district Kohistan is located on the north. The forest covers 8,090 acres, with an average rainfall of 1778 mm while monthly is 150 mm, maximum snowfall of 60 inches from November to March, a maximum temperature is above 25°C in June and July, and a minimum up to 0°C in December. The

7**plant name has been verified (http://www.theplantlist.org/) and**

the taxonomy of the plant according to National Plant Data Center, NRCS, USDA is as follows.

55**Kingdom: Plantae Sub-Kingdom: Traciobionta Division: Pteridophyta Class: Filicopsida Order**: Polypodials **Family: Pteridaceae Genus: adiantum. L**

Species:

83**Adiantum capillus- veneris Linn Adiantum capillus- veneris Linn**

(Ahmed et al., 2012) Aim and Objectives This experiment aims to investigate the protective effects of plant Adiantum capillus-veneris Linn. against toxicity that furan caused in adult male Sprague Dawley rats. ? To evaluate testosterone levels in blood plasma through ELISA. ? To measure stress by cortisol ELISA ? To evaluate lipid profile in blood plasma through a chemistry analyzer. ? To determine the effect on sperm parameters of rat testis. Materials and Methods The study

77**was carried out at Quaid-i-Azam University**

, Department of zoology Reproductive Physiology Laboratory, in Islamabad. The local

43**ethical committee of the Department of**

zoology approved guidelines and regulations for animal handling and management. Chemical

8**Furan (CAS 110**-009, **Sigma Aldrich Co. Ltd, Sigma -Aldrich Poole, Dorset**,>99%**pure) was** made **in corn oil on the day of** usage **and stored in sealed brown bottles**. For 28 days, **furan was** administrated **orally** through **gavage in corn oil at**

a dose of 40mg/kg. 240mg/kg of methanolic leaf

92**extract of** Adiantum **capillus-veneris** L. **was** given. Based **on**

previous findings of furan stability, doses were made separately in a volume-to-weight ratio (v: w) and stored in the refrigerator (sealed with plastic closure and modified silicon septa) for 14 days (NTP 1993). After 14 days, the same protocol was followed once more. Before being given to animals, furan was dissolved in corn oil, and plant extract was dissolved in normal saline. Animals Twenty adult Sprague Dawley male rats (Rattus norvegicus), weighing of 150±10g, were taken from

53**Quaid-i-Azam University Islamabad** Zoology Department. Rats **were** separated **into four groups**

at random and

53**kept in** separate **stainless-steel cages and**

the normal

20**colony conditions were maintained (Ullah et al., 2016**) The Ethical Committee **of**

the Zoology Department approved animal handling and experimental procedures. Plant Material Identification of the leaf sample of Adiantum capillus-veneris Linn was done

> 7**by Dr. Mushtaq Ahmad at Plant Systematics and Biodiversity** Lab **and Herbarium of Pakistan, Quaid-i-Azam University Islamabad**. This **plant**

was collected from agricultural and cultivated fields of Alpuria, Chakesar, Hayatabad, Shangla, Lilowni, Ajaori, Kass, and Shahpur, Pakistan. Plant Extract Preparation

> 75**Adiantum capillus-veneris** Linn **leaves were** detached **from the** stem and dried **in**

air. These leaves of the plant that dried in air were stored until the extract was made that weigh about 2 kg. The leaves were crushed and sieved in a Waring blender. By using methanol, leaves dried powder according to this ratio (leaves to solvent ratio 1:10) was extracted. Whatman filter paper is used for filtration of the plant extracts and concentrated on a rotary evaporator (Gulfraz et al., 2007). Experimental Design For the present

> 86**study, the animals** (n=5) **were divided into** four **groups**. All **the**

doses were administrated orally between 10-11 am for consecutive 28 days. Group I This group was treated with 0.9% N. saline and considered a control group. Group II Group II rats were provided with 40mg/kg of furan and were prepared in corn oil. Group III Methanolic leaf extract dissolved in saline was given to Group III animals at a dose of 250mg/kg. Group IV Group IV animals were given 40mg/kg of furan dissolved in corn oil and 250 mg/kg of methanolic leaf extract dissolved in saline. The final

> 82**body weights of all** the **animals were measured** on day 29 **and**

the animals were decapitated. Figure 4: Experimental Design Blood and Tissue collection The experiment was carried out for 28 days. The animals were weighed and decapitated on day 29. Heparinized syringes were used to collect trunk blood directly after decapitation and kept in heparinized tubes. For 15 minutes, centrifuged blood samples at

> 85**3000 rpm. Plasma was isolated and** kept **at** -20˚**C**

till it came time to study it. Both testicular and epididymal tissues were obtained from all the animals. Analysis for daily sperm production, ice-cold saline was used for cleaning of left testis and was stored in the freezer. The viability and motility of sperm and epididymal sperm count was determined from the left epididymis after washed and weighed, then minced for more assessment. On testis and epididymis (right), the histological analysis was done that were fixed in 10% formalin. Sperm Parameter: Assessment of Sperm Motility In normal saline solution (1ml), a small cauda portion of the epididymis was cut and placed at 37˚C and crushed for assessment of sperm motility. On warmed slides homogenate sample (10µl) was placed by using pipette. A 10 fields minimum were examined under high power microscopy (40× magnifications) and 100 spermatozoa were (Halvaei et al., 2012). Assessment of Sperm Viability For determination of viability of sperm, an nigrosine-eosin test was used. The dye (eosin- nigrosine) of about 25µl were mixed with samples of semen. Smear was made by placed on a glass slide,15µl of this mixture, and at room temperature slides was dried. Later, by using a light microscope (40× magnification) was used for the examination of these

slides. The spermatozoa that remained unstained (white) are alive whereas, stained red spermatozoa are dead. At least 100 sperm cells were counted and obtained the dead and alive spermatozoa percentage (Halvaei et al., 2012). Daily Sperm Production (DSP) At room temperature, thawed testis tissues (frozen)

> 2**for a few minutes** before **the homogenization process. Spermatids** that **were** unaffected by **homogenization (19th stage of** spermiogenesis) **in the** homogenate, **were counted by the method followed by Robb** et al. (**1978); Tunica albuginea was removed, and then parenchyma was weighed homogenized** for 30 seconds **in** 3ml **of**

0.9% (NaCl) containing 100 X 0.5% triton and then homogenized (Robb et al., 1978). The dilution of the resulting homogenate was made up to 5 folds. In Neubauer's chamber, 20µl of the sample was deposited and by using a microscope (x40 magnification), late spermatids were counted. To determine the spermatids number, for each sample at least three readings were taken. From this reading the spermatids total number per testis was calculated, then divide by weight of testis to get the spermatids number. That considered the capability of sperm production. The spermatids that were non- effective against homogenization process was divided by 6.3, for the calculation of DSP, which characterizes the spermatids stayed in the seminiferous epithelium for days. Daily Sperm Production formula Daily sperm production formula Y = No. of sperms (N) ×25×1000×5×25* = Y/6.3 Were, Y= No. of spermatids present in homogenate N = Total no. of spermatids in Neubauer's chambers are counted. 25= Total no. of squares in the chamber. 5 = With physiological saline dilutions made. 1000 = to convert µl into ml 25*= dilutions made with PBS

> 42**Daily sperm production (DSP) = Y/6.3** Biochemical **Analysis**

: Serum biochemistry analysis Using a chemistry analyzer (AMP diagnostic) and AMP diagnostic kits (AMEDA Labordiagnostik GmbH, Austria) and, TC (Cat # REF10498999318389), TG (Cat # REFBR4501),

> 70**High-density lipoprotein** (Cat # 104989993194), **and Low-density lipoprotein** (Cat # BR3302) **were measured in**

blood plasma obtained from the experiment as given in handbook of instruction. 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 microwell plates with goat anti-rabbit to form solid-phase antibodies. Add testosterone antibody, testosterone calibrator, and HRP -testosterone to form a secondary antibody. The binding amount of HRP-testosterone is reversely proportional to testosterone content 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: ? Microtitration 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 microtiter 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 incubated at 37°C for about one hour. Finally added 50µl stop solution to each well and mix completely. ? Read the absorbance of the plate within 10 minutes. 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. To the wells coated with streptavidin, the samples, a working solution of the

> 25**cortisol-HRP conjugate, and** a solution of **anti-cortisol-biotin are added**

. For binding sites,

> 25**cortisol in the patient's serum competes with the cortisol enzyme (HRP**

)

> 14**conjugate. Washing buffer** removed **unbound** cortisol **and** cortisol **enzyme conjugate., The** concentration **of** cortisol **is inversely** proportional **to the** intensity **of** the color **in samples** upon **addition of substrate. A standard curve linking color intensity to** the **concentration** of cortisol was **created. Procedure**

: Reagents are

> 28**allowed to stand at room temperature**

, prior to starting of assay Placed the

> 28**desired number of coated strips into the holder**. ? Then added **25µl of** cortisol standard, **control, and patient sera**

. 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. then

> 39**added 100µl** of **TMB substrate and incubated for** 15 **minutes at room temperature**. ? Finally **added 50µl stop solution** to all wells **and** mixed completely. ? **Read the**

absorbance at 450 nm within 20 minutes. Statistical Analysis Graph pad Prism software was used to perform a

> 40**one-way analysis of variance (ANOVA) followed by** a **Post-hoc Tukey's test to compare** different **groups. All** the results **were**

> 54**presented as Mean ± SEM. The significance level** was **set at p < 0.05. Results** Bodyweight: **In** comparison with **the**

control group, the furan

> 26**treated group** had **a highly significant** decrease (**p<0.001) in body weight

was detected. In ACV (p=0.90) and ACV +Furan (p=0.41) treatment groups, non-significant increase in body weight was seen in comparison

> 80**with the control group**. In comparison **with the** furan **group, the**

ACV and ACV+ Furan treatment groups had

> 26**a highly significant increase (p<0.001) in body weight**

was observed. However, non- significantly decrease (p=0.48) was evident in the ACV+ Furan treated group in comparison with the ACV group. Table: 1 Mean ± SEM body weight gain (g) among adult Sprague Dawley male rats following treatment furan and ACV dosage. Parameter Control Furan ACV Furan + ACV P value statistics Body weight gain (g) 41.17±4.38 21.09±3.02a*** 43.56±1.08b*** 42.06±4.21b** 0.002 *

> 1**p< 0.05, **p< 0.01, ***p< 0.001, values are stated as mean**

± SEM showing significant variance respectively. a value used in the comparison of control, while b value used in the comparison of furan and c value used in the comparison of ACV Testicular weight:

> 6**When compared to** the **control group highly significant decrease (p<0**.001) **was** observed **in**

weight of both testis (right, left) in

> 52**rats treated with** furan. **When compared with control group**, non-**significant**

increase in weight of both testis (right, left) among ACV (p=0.70) and Furan +ACV (p=0.70) treatment groups was observed.

> 6**A significant** increase (**p<0.05) in weight of**

both testis (right, left) among ACV and Furan +ACV groups in comparison to the furan group. In Furan +ACV group, no significant change (p=0.79) was observed

> 4**as compared to the** furan **group**. Epididymis Weight: **A** highly **significant increase (p<0**.001) **in**

both right and left epididymal weight was seen in rats treated with furan, in comparison to control group. While, in comparison with control, a non-significantly increase among ACV and Furan+ ACV groups was

noticed. However, no significant change (p=0.60) was observed in both right and left epididymal weight among ACV and Furan +ACV groups as compared to the furan group as well as the ACV group. Accessory Organs Weight:

> 36**When compared** to **control group, a** remarkable **decrease (p<0.001) in** weight **of**

prostate and seminal vesicle in rats treated with furan was observed. While non significantly increase (p=0.59) in prostate and seminal vesicle weight was evident among ACV and Furan +ACV groups as

> 58**compared to** the **control group. In** comparison to **the** furan group, **a** highly **significant increase (p<0.001**) in prostate **and**

seminal vesicle weight was seen among ACV and Furan +ACV treated groups. Table:2 Mean ± SEM Testicular weights (g), Epididymis weights (g), and accessory organ weight (g) among adult male Sprague Dawley rats following treatment with furan and ACV dosage. Parameters Control Furan ACV Furan + P value ACV statistics Testis weight (R) 1.43±0.02 1.32±0.05a*** 1.45±0.02 b* 1.44±0.02 b* 0.009 Testis weight (L) 1.42±0.02 1.30±0.03a*** 1.43±0.05 b* 1.42±0.02 b* 0.02 Epididymis (R) 0.12±0.07 0.66±0.01a*** 0.71±0.06 a*** 0.62±0.03a*** 0.001 Epididymis (L) 0.56±0.12 0.64±0.10a*** 0.70±0.05a*** 0.60±0.02a*** 0.68 Prostate gland 0.65±0.01 0.50±0.

> 79**01a*** 0.67±0.01b*** 0**.66±**0.01b**

*** 0.001 Seminal Vesicle 0.74±0.01 0.40±0.03a*** 0.78±0.01b*** 0.77±0.02b*** 0.001 *

> 1**p< 0.05, **p< 0.01, ***p< 0.001, values are stated as mean**

± SEM showing significant variance respectively. a value used in the comparison of control, while b value used in the comparison of furan and c value used in the comparison of ACV Sperm Motility In comparison with the control, remarkable decline in sperm motility rate was evident in the furan group (p<0.001). Non-significantly increase was evident in rate of sperm motility among ACV (p=0.98) and Furan+ ACV(p=0.25) groups in

> 35**comparison with the control group**. There **was** a **significantly increased (p<0.01**

) in sperm motility rate among ACV and Furan+ ACV groups in comparison

> 9**with the** furan **group. However**, no **significant** change (**p=0**.25) **in**

sperm motility rate

> 9**was observed in the** Furan+ ACV **group** in comparison **with the** ACV **group**

. Sperm Viability:

37**There was a** prominent **decrease in** sperm viability rate **in the** furan **group**

(

13**p<0.001**) observed **in comparison** with **the control group**. No **significant** increase (**p=0**.26) was seen **in**

sperm viability rate

37**in the** ACV **group** as **compared with the control**. When **compared** to **the control**

, a prominent increase in rate of

20**sperm viability (p<0**.05) **in the**

ACV group was noticed. A significantly decreased sperm viability rate was observed in Furan+ ACV groups (

6**p<0.05) in comparison with the control group**

as well as the ACV group.

4**Daily Sperm Production: In** comparison to control **group**, a **in**

daily sperm production rate is remarkable reduce in rats treated with furan (p<0.001) was observed. There was a non-significantly increase observed in daily sperm production among ACV (p=0.56) and Furan +ACV (p=0.19) groups in comparison with control group. In ACV and Furan + ACV treated groups (p<0.01) a significant rise in daily sperm production were noticed in comparison with furan group. While non significantly decrease (p=0.004) was evident

4**in daily sperm production in** Furan +ACV groups **in comparison with**

furan group. Table 3: Mean ± SEM sperm parameters among adult male Sprague Dawley rats following treatment with furan and ACV dosage. Parameters Control Furan ACV Furan + ACV P value statistics Sperm motility (%) 62.2±4.07 42.3±2.59a*** 65.30±2.59b** 63.2±2.56b** 0.001 Sperm viability (%) 82.2±2.46 62.2±4.07a*** 84.30±2.49b* 83.3±2.51a*b* 0.001 Daily sperm production×106/Testis 2.23±4.47 1.69±1.33a*** 2.26±8.54 b** 2.25±1.31 b** 0.001 *

1**p< 0.05, **p< 0.01, ***p< 0.001, values are stated as mean**

± SEM showing significant variance respectively. a value used in the comparison of control, while b value used in the comparison of furan and c value used in the comparison of ACV Figure 05: Mean ± SEM % rate of sperm motility among adult male rats following treatment to furan and ACV dosage. Figure 06: Mean ± SEM % rate of sperm viability rate among adult male rats following treatment to furan and ACV dosage. Figure 07: Mean ± SEM daily sperm production among adult male rats following treatment to furan and ACV dosage. Lipid Profile: Cholesterol: Significant elevations were observed in the cholesterol levels among the furan-treated group, ACV group (p<0.001), and Furan + ACV group

62**treatment group (p<0.001**) when a **compared with the control group**. When **compared with** control **group**

, a highly significant decline in the levels of cholesterol among the ACV group and Furan + ACV treatment groups (p<0.001) was noticed. When a comparison was made with the ACV group, a prominent elevation in the levels of cholesterol in Furan + ACV group (p<0.001) was noticed. Triglycerides:

32**A significant increase was seen in** triglycerides level in **the** furan treated **group (P<0.05**) when **compared** with **the control group**. While **a significant decrease in the**

level of triglycerides was evident

61**in the** ACV **group (P<0.05**) when **compared with the** furan **group**. However, **there was** no **significant**

change was experienced among the ACV (p=0.80) and Furan + ACV (p=0.60) groups in comparison with the control and furan groups. High-density lipoprotein (HDL): A remarkable

47**decrease (P<0.001) in** HDL levels **was** evident **in** the furan **group**

in comparison with control.

88**There was a** non- **significant increase (p=0**

.09) seen among ACV and Furan + ACV treated groups in comparison with the control. While, in ACV group and Furan + ACV

34**group (P<0.001), a significant** rise **in the levels of**

HDL was evident

81**when compared with the** furan **group**. Non-significantly increase (**p=0**.19) **was**

evident in Furan + ACV treatment groups when comparison made with the ACV group. Low-density lipoprotein (LDL): In furan treated group, a remarkable

36**increase (p<0.001**) was detected **in levels of** LDL **in**

comparison with control group. While in the ACV group, a non-

13**significant decrease (p=0**.10) was experienced **in comparison** with **the control group**. A **significant decrease (p<0.001**) was observed **in** LDL **levels** in Furan + ACV **group**

when a comparison was made

57**with the control group. A** prominent **decrease**

(

4**p<0**.001) **in the level of** LDL **was observed in** ACV as **compared to the** furan **group. However**

, cortisol levels in the Furan+ ACV groups

45**were found to be** decreased non-**significantly (p=0**.19) than **in** furan **group**

. When compared to ACV group, significant elevation in levels of LDL in the Furan + ACV treated group (p<0.001) was noticed. Table: 4 Mean ± SEM Cholesterol, triglycerides,

72**high-density lipoprotein, and low- density lipoprotein concentration (mg/dL**

) among adult male Sprague Dawley rats following treatment with furan and ACV dosage. Parameters Control Furan ACV p value Furan + ACV statistics

66**Cholesterol (mg/dL) Triglycerides (mg/dL) HDL (mg/dL) LDL (mg/dL**

) 143.56±3.63 286.16±3.93a*** 100.43±1.28 60.59±2.25 48.05±0.72 159.13±14.9a* 38.56±3.43a*** 93.35±2.12a*** 184.93±4.44ab*** 106.09±2.02 b* 62.43±3.43 b*** 41.66±1.61 b** 236.30±5.90abc*** 116.00±4.81 67.93±2.25 b*** 46.85±1.06 ac** 0.001 0.05 0.001 0.001 *

1**p< 0.05, **p< 0.01, ***p< 0.001** these **values are stated as mean**

± SEM showing significant variance respectively. a value used in the comparison of control, while b value used in the comparison of furan and c value used in the comparison of ACV Figure 08: Mean ± SEM cholesterol levels (mg/dL) among adult male Sprague Dawley rats following treatment with furan and ACV dosage. Figure 09: Mean ± SEM triglycerides levels (mg/dL) among adult male Sprague Dawley rats

following treatment with furan and ACV dosage. Figure 10: Mean ± SEM HDL levels (mg/dL) among adult male Sprague Dawley rats following treatment with furan and ACV dosage. Figure 11: Mean ± SEM LDL levels (mg/dL) among adult male Sprague Dawley rats following treatment with furan and ACV dosage. Hormonal Analysis: The concentration of testosterone significantly reduced in rats treated with furan (

7**p<0.001) and** non-significantly increase (**p=0**.49) **in levels**

of testosterone was evident among ACV and Furan+ ACV groups when comparison made with the control

9**group. When compared with the** furan **group, a highly significant increase (p<0.001) in** levels **of**

testosterone among ACV and Furan+ ACV treatment groups was observed. However, a non-significantly rise (p=0.17) was seen in levels of testosterone in the Furan+ ACV treated group in comparison with ACV group. A remarkable increase in levels of cortisol was detected in furan treated rats (p<0.001) in

35**comparison with the control**. There **was** non-**significantly** rise (**p=0**

.11) in concentration of cortisol in ACV in comparison

45**with control group While, significant** increase **was observed in**

cortisol levels in Furan+ ACV

34**group (p<0.05**) as **compared to the control group**. Cortisol levels **in**

ACV

2**were significantly decreased (p<0.05**) when compared with **the** furan **group**. However, **when compared to the** furan group, levels **of**

cortisol were

47**found to be** non-significant **decreased (p=0**.80) **in** Furan+ ACV **group**

. When comparing the Furan+ ACV groups

6**to the** ACV **group, there was a** non- **significant** increase **in** cortisol **levels**

(p=0.50). Table 5: Mean ± SEM plasma levels of testosterone and cortisol (ng/mL) among adult male Sprague Dawley rats following treatment with furan and ACV dosage. Parameter Control Furan ACV Furan + ACV P value statistics Testosterone (ng/mL) Cortisol (ng/mL) 4.16±0.29 75.4±4.41 2.66±0.22 a*** 4.46±0.32b*** 112.1±7.27a*** 90.1±4.42b* 4.66±0.31b*** 96.08±7.44a* 0.001 0.006 *

1**p< 0.05, **p< 0.01, ***p< 0.001** these **Values are stated as mean**

± SEM showing significant variance respectively. a value used in the comparison of control, while b value used in the comparison of furan and c value used in the comparison of ACV Figure 12: Mean ± SEM Testosterone levels (ng/mL) among adult male Sprague Dawley rats following treatment with furan and ACV dosage. Figure 13: Mean ± SEM cortisol levels (ng/mL) among adult male Sprague Dawley rats following treatment with furan and ACV dosage. DISCUSSION Furan is a highly volatile heterocyclic organic molecule.

44**It has been classified as a probable human carcinogen**

(IARC 1995; NTP 1995). Recently, furan has been discovered in many heats treated foods, including vegetables, baby foods, sauces, canned and jarred, and soups (US FDA 2004). As a result, the existence of furan in various items such as baby foods, cereals and coffee may

48**have a** considerable **impact on overall human to** furan exposure, necessitating **a**

greater understanding of the health concerns linked with their intake (Rahn & Yeretzian, 2019). Recently reported that reproductive problems by disturbing spermatogenesis caused by furan exposure, therefore, lead to cell death in germ and Leydig cells. Thus, furan is of high concern on a global scale, because of its adverse effect on mammals including humans (Hamadeh

15**et al**., 2004). **A** remarkable **number of** modern drugs **have been developed and**

isolated from plant sources that are known to be effective against chemicals that act as endocrine disruptors (Mustapha, 2013). Because of the therapeutic and nutritional value of Adiantum capillus-veneris L., it was used as herbal medicine (Al-Snafi, A. E. 2015). Therefore, the current study aimed to determine the protective

24**effects of Adiantum capillus- veneris** L. against **toxicity** caused **by** furan in **adult male** Sprague Dawley **rats**

. To conduct a present

12**study, adult male rats were orally** administrated **with** 40mg/kg **of**

furan and 250mg/kg of methanolic leaf extract of Adiantum capillus-veneris L. for 28 consecutive days.

10**Body mass index (BMI**) is **used to assess overweight/obesity** index **and to monitor changes in body weight. Measurements of body weight alone can be used to determine** the **efficacy of weight loss therapy**

(Blackwell, 2002). In current study, results showed body weight gain and weight of accessory organ decrease significantly in furan group in comparison to control group. Our results are following previous

studies conducted by Rehman et al.(2019), where body weight and accessory organ weight among furan administered group (40mg/kg) showed a decrease in body weight and accessory organ weight (Rehman, Jahan, et al., 2019). In rats, oral administration of furan is capable of easily passing through a biological membrane and is rapidly absorbed from the intestines, however, primary targets of toxicity of furan is liver, where P-450 cytochrome enzymes rapidly metabolized furan to form cis-2-butene1,4-dialdehyde (BDA) and CO2, and BDA is the main metabolite, and it is considered cytotoxic and irreversibly bind to nucleosides and proteins (Hamilton et al., 2006; Chapin et al., 1997). The underline pathway by which furan impacts body weight gain is not known, however, it could be an indirect effect seen by changes in plasma hormones or direct effects on food intake (Mårin et al., 1992). Further, in ACV treated group (250mg/kg) body weight gain and weight of accessory organs is non- significantly increased was detected in comparison with control, as supported by previous studies by Gaikwad et al. (2013), where higher doses of ACV (500mg/kg) showed a remarkable increase in body weight in rats (Kanchan Gaikwad, 2013). Body weight is found to increase non-

38**significantly in the ACV** + furan treatment **group in comparison to control**

was observed. Our results are similar to the previous findings, where decreased body weights following bisphenol A and cisplatin exposure was restored by the protective effects of Adiantum capillus-veneris L. in rats (Kanchan Gaikwad, 2013; Yousaf et al., 2016). For normal fertilization sperm motility is necessary and for the evaluation of ejaculated sperm's fertilizing capacity, sperm motility is considered the most important factor. In the present study, viability, and motility of sperm and DSP rate is found to decrease significantly was noticed in the furan treated group (40mg/kg) in comparison to the control group.

50**A similar result was also** previously published **by** (Rehman, Jahan, **et al**

., 2019), where a decrease in sperm viability and motility after furan exposure was noticed. Similar observations were stated by Uzunhisarcikli et al. (2007), where a reduction in sperm number as a result of a decrease in levels of testosterone. Recently, literature also reported that spermatozoa exposed to high levels of reactive oxygen species resulting in decreased viability and motility of sperm, which leads to infertility, however cell-to-cell interaction and spermatogenesis and within the testis controlled by testosterone (Aitken et al., 2011). The result of the present study could be explained by the lower levels of testosterone. As the normal concentration of testosterone is required for the normal structure maintenance and accessory sex organs functions, a decreased in the rate of DSP can be caused by decreased levels of testosterone in the blood (Sethi et al., 2010). The increased levels of cortisol might be lead to suppression of spermatogenesis and cause disruption in

43**spermiation, and impairment of sperm quality** (Pressman **et al**., 2018; Castranova **et al**., 2005). Long **exposure**

to furan was linked to cell death in Leydig cells and germ cells and as well as lower LH and testosterone levels, according to previous research (Karacaoğlu et al., 2010). Further, non-significantly increase in sperm viability motility and daily sperm production in ACV (250mg/kg) treated group was observed as

42**supported by a previous study conducted by** (Yousaf **et al**

., 2016) was showed that plant Adiantum capillus-veneris L. might be androgenic effects by increasing sperm

78**viability, motility**, and **daily sperm production. Sperm** viability, motility, **and** daily **sperm**

production also non significantly increased in Furan + ACV treated group

33**as compared to control group. A similar result was** also previously published **by**

(Yousaf et al., 2016), where decreased sperm viability motility and daily sperm production following bisphenol A exposure was restored by the protective effect of plant Adiantum capillus-veneris L. in rats. A lipid profile is a combination of blood tests that help in determining the quantities of lipids, such as cholesterol and triglycerides present in the bloodstream. (Ngala et al., 2018). In different body organs and cells, lipids perform vital functional and structural roles as well as maintain the normal body processes (Rawi et al., 2012). The present study result indicated a highly significant increase in plasma cholesterol, LDL, and triglycerides levels, while HDL was decreased in furan group (40mg/kg) in comparison to the control.

56**These findings are in** line **with** the earlier studies, **conducted by** (Rehman, Jahan, **et al**

., 2019), where after furan exposure, levels of plasma triglyceride, total cholesterol,

33**and LDL were** elevated, **while the levels of HDL were** decreased. Previous literature **also**

reported that oxidative stress induces aberrant serum lipid concentration and lipid profile disturbance in the bloodstream commonly known as dyslipidemia (Martins et al., 2018). Recently, literature also reported that increased fatty acids production causes a decrease plasma HDL level, and a rise concentration of plasma cholesterol concentration, which can lead to liver dysfunction. Because normal levels of HDL and LDL are indicators of proper functioning of the liver. In a clinical test, a ratio of TG/HDL levels is used for detecting people who appear to be healthy individuals but have cardiovascular and metabolic impairments (Raju et al.,2011; Ghanayem et al.,2010). However, after furan administration, the current study found reduced HDL levels, it might be explained by the fact that during early phases of development, plasma lipoproteins levels are low (Brai et al., 2020). Another description for the detected change in levels of plasma HDL and LDL is that the release of hepatic triglycerides into the blood is inhibited by different liver toxins (Koszucka et al., 2020). For example, acrylamide, cause changes in the same way (Ghanayem et al.,2010.; Raju et al.,2011). It can explain that observed low triglycerides levels that may lead to an accumulation of triglycerides in the liver, which caused fibrosis and liver damage in overweight rats (Rehman, Jahan,

41**et al**., 2019). **The** result **of the present study** might be similar **to the**

observation stated by Ldeniz et al. (2011) that LDL receptors overactivation might be caused due to increased levels of plasma LDL. In later findings, that furan exposure in male rats had elevated LDL levels in blood plasma and LDL receptors assist the entry of cholesterol molecules in the normal human body cells. The low-density lipoproteins release their cholesterol and triglycerides on attachment to their receptors on the hepatocytes (Shah et al., 2022). Because of elevated levels of cholesterol, caused by decreased low-density lipoproteins transport into cells, the formation of new LDL receptors was ceased. Limiting the LDL

intake and the non-functional receptors improves the serum cholesterol levels (Aldred, 2008). Increased free cholesterol levels prevent the synthesis of the receptor of LDL, thus more accumulation of cholesterol is promoted by less intake of LDL (M, 2001). Previously, researchers have also reported that low levels of testosterone, abnormalities of sperm, and male infertility might be linked to increased cholesterol and reduced HDL levels (Shalaby et al., 2004). However, the result of the present study in ACV treated group (250 mg/kg) decreased levels plasma cholesterol levels, and LDL triglycerides, while

19**an increase in levels of** HDL **was observed in**

comparison to the control group;

4**these findings are** similar **with previous studies**

where, high cholesterol diet-fed rats exposed to Adiantum capillus- veneris L. results showed the total cholesterol (TC), LDL, and triglycerides levels were all reduced but did not affect HDL levels, because

23**Adiantum capillus-veneris L**. exhibited hypocholesterolemic property. (**Al-Hallaq et al., 2015**

). Plasma cholesterol LDL and triglycerides levels were also decreased while HDL levels were also increased in the ACV+Furan treatment group, these findings are following previous studies conducted by (Kanchan Gaikwad, 2013; Yousaf et al., 2016) significantly decreased serum LDL levels and increased HDL level following bisphenol A and cisplatin exposure was restored by protective

23**effects of Adiantum capillus-veneris** L. **in** rats. The maturation **and**

development of spermatozoa and normal steroidogenesis are important for male infertility. Normal production and release of gonadotrophin (LH, FSH) are controlled by the phenomena of spermatogenesis and steroidogenesis in testes (Rehman, Ullah, et al., 2019) The normal secretion of testosterone is under the control of luteinizing hormone The appropriate concentration of testosterone is important for the normal function of the testes. In the current study, the hormonal concentration of plasma testosterone was significantly reduced when rats were treated with furan (40mg/kg) in comparison to the control. Formerly, similar results have been reported that reduced testosterone levels after exposure to the higher dose of furan (Rehman, Ullah, et al., 2019). However, reduced testosterone levels are a sign of biochemical toxicity (Yoshida et al., 2002). Recently, literature reported that oxidative stress might be caused decreased levels of testosterone and increased spermatogenesis sensitivity, which is closely associated with primary functions of antioxidant enzymes in Leydig cells (

20**Cao et al**., 2008; **Rezvanfar et al.,2013). The present study**

results can be explained that normal function of Leydig cells could be affected after furan exposure, result decreased testosterone levels. The reduction in T concentration is significantly contributed by the reduction in luteinizing hormone in male rats which encourages spermatogenic arrest and infertility. Non -significant increased testosterone concentration in ACV treated group (250mg/kg) was observed when a comparison was made with the control group. The result of present study was similar to previous study shown by Tawab et al. (2014), which stated that for the treatment of uncontrolled ejaculation, Adiantum capillus-veneris L. is

used as an indigenous or folk medicine. Moreover, its ability to increase testosterone levels in blood prove its protective effects on the male reproductive system. Testosterone concentrations were

38**increased in** Furan + **ACV** treated **group in comparison** with **the control group**

. Similar findings were detected by Yousaf et al. (2016), where following exposure to bisphenol A, testosterone concentration was restored by protective

23**effects of Adiantum capillus-veneris** L. **in** rats. **In**

the current study, increase cortisol levels were detected in the animals of furan treated group (40mg/kg) when a comparison was made with the control group. These findings are under earlier reports of increased cortisol levels after furan exposure (Rehman, Ullah, et al., 2019). The increased cortisol levels have been linked with a reduced synthesis of growth hormones and sex steroids in multiple findings (Liening et al., 2010; L. J. Chen et al., 1997). PCBs, as well as heavy metals are endocrine disruptors that might be cause an increase levels of cortisol in mammals and fish (Tort

74**et al., 2011**; Sumera **et al**., 2018; Tan **et al., 2007**

). Previously reported that elevated cortisol causes energetic costs of reproduction (Katrine Knutsen et al., 2017; Leal et al., 2011). Previous findings reported that the higher levels of cortisol might be used to determine the disturbance in growth of reproductive organs caused by reduced levels of testosterone. No significant rise in cortisol levels was detected in ACV treated group (250mg/kg) in comparison with the control group. There is no previous literature available about the

24**effect of Adiantum capillus-veneris** L. **on**

cortisol levels. A

91**significant increase in** cortisol **levels was** seen in **the**

Furan+ACV treatment

41**group as compared to the control group. There** is **no**

previous literature available on the protective

24**effect of Adiantum capillus-veneris** L. **on**

cortisol levels. Conclusion

73**The results of the present study showed that** a high concentration **of**

furan can have antiandrogenic

12**effects on the** genital **system in** adult male **rats**

resulting in

12**a decrease in** body **weight** gain, lowering **sperm motility**

, viability, and daily production rate, increase in cholesterol, triglyceride,

64**low-density lipoproteins (LDL) and decrease** in **high-density lipoproteins (HDL**), as well as **a** reduction **in**

testosterone and increase in cortisol levels, later restored by Adiantum capillus-veneris L.

18**To understand the** underlying **mechanism of action of** furan **in**

reproduction at a cellular and molecular levels, further studies can be planned.