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Protective effects of alcoholic extract of *Tribulus terrestris* against Cytarabine induced hepatic and testicular dysfunction in male rats

Submitted to the Council of the College of Veterinary Medicine, University Of Kerbala in Partial Fulfillment of the Requirements for the Master Degree of Science in Veterinary Medicine / Physiology

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بسم الله الرحمن الرحيم

) يَٰٓأَيُّهَا ٱلَّذِينَ ءَامَنُوٓاْ إِذَا قِيلَ لَكُمۡ تَفَسَّحُواْ فِي ٱلۡمَجَٰلِسِ فَٱفۡسَحُواْ يَفۡسَحِ ٱللَّهُ لَكُمۡۖ وَإِذَا قِيلَ ٱنشُزُواْ فَٱنشُزُواْ يَرۡفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمۡ وَٱلَّذِينَ أُوتُواْ ٱلۡعِلۡمَ دَرَجَٰتٖۚ وَٱللَّهُ بِمَا تَعۡمَلُونَ خَبِيرٞ)

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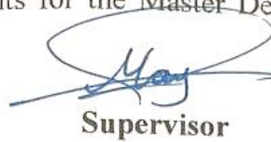

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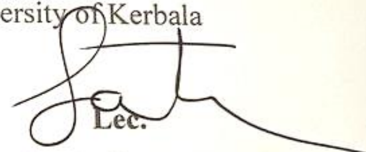

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/ / 2024

Dedication

To the one who fed me, watered me, and showered me with the abundance of grace, my creator and my beloved.

To the one whose birth was a mercy to the worlds, and the ships of salvation, the Messenger of Allah, Muhammad, and the Allah of Purity, is my support.

To the heart that overwhelmed me with love and tenderness, my father, may God prolong his life, and my mother.

To my support and my strength in life, the companionship of my life, and my path towards the future my dear husband, may God prolong his life

To the apple of my eye and my open flowers, my children

To my arms, my loved ones, my dear brothers and sisters

To the pure souls and souls that surrounded me and my honorable family

To the one who removed the fog of ignorance from me with the light of his knowledge and illuminated my path with the light of his knowledge from my teachers

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And to everyone who is interested in my success, I dedicate my humble effort

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Summary

The present study investigated the possible therapeutic protective, the efficacy as an antioxidant, and the protective effects against chemically induced reproductive dysfunction on spermatogenesis damage. The cytotoxic effects of numerous cancer treatments can lead to various general adverse consequences and impairments in spermatogenesis. Therefore, it remains an important concern that need further improvement. Cytarabine (Ara C), a widely used chemotherapy drug for hematological malignancies, exerts detrimental effects on male reproductive development and function by suppressing germ cell proliferation and inducing apoptosis. Twenty mature male rats will be divided into four groups. Each group contains 5 rats. The first group is a control group, the second group i.p ingection with Ara-C (25 mg/kg BW) and the third group is oraly with T. Tribulus terrestris extract (250 mg/kg) and fourth group is dosed with Ara-C (25 mg/kg BW) intraperitoneally + *T. terrestris* extract (250 mg/kg bw) orally for 28 days. The HPLC examination of the dry extract of TTE revealed the presence of a variety phenolic compounds. Our work aimed to investigate TTE protective function against Ara C-induced reproductive impairment in male adult rats. Ara C caused dose-dependent increase in serum liver enzymes activities (AST, ALT and ALP); elevated oxidative stress; impeded steroidogenesis (lower testosterone, follicle-stimulating hormone, luteinizing hormone. Furthermore, immediate exposure to Ara-C resulted in a decrease in 17 β-HSD activity, could be linked to the decrease in testosterone concentration in the blood, the reported histological abnormalities in the testes, and the quality of the semen, and raised caspase concentration as compared with other groups.

In conclusion Ara-C that revealed detectable spermatogenesis degeneration and causes testicular damage, and *T. terrestris* works to reduce cytarabine damage. The current investigation emphasized the importance of TTE in improving the changes caused by Ara-C injection in the testes of rats, perhaps due to the presence of antioxidant compounds.

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List of abbreviations

Abbreviations	**Meaning**
ABC	ATP-binding cassette
ALP	alkaline phosphatase
ALT	alanine amino transferase
AML	acute myeloid leukemia
Ara C	Cytarabine
Ara-CMP	Ara-C monophosphate
AST	aspartate amino transferase
17 β-HSD	17β-Hydroxysteroid dehydrogenase
CAT	Catalase
CDA	cytidine deaminase
GI	Gastro-intestinal
CP	Cyclophosphamide
CREMs	CAMP-responsive element modulators
CYP	cytochrome P3A450
DCK	like deoxycytidine kinase
dCK	deoxy-cytidine kinase

DCMD	deoxycytidyne monophosphate deaminase
DNA	deoxyribonucleic acid
FSH	follicle-stimulating hormone
GnRh	Gonadotrophin revealing Hormone
H&E	Hematoxylin and Eosin stain
i.v.	intravenous
LH	luteinizing hormone
MDA	malondialdehyde, and
mg/dl	Milligrams per deciliter
mg/kg	Milligrams kilogram
NAD	nicotinamide adenine dinucleotide
NADPH	Nicotinamide Adenine Dinuc
NDK	nucleoside diphosphate kinase
ROS	reactive oxygen species
SLC	solute carriers
SOD	superoxide dismutase
SS	secondary spermatocytes
TOS	Total oxidant status

TTE	*Tribulus terrestris* extract
WBC	white blood cells
DHEA	Dehydroepiandrosterone
NO	Nitric oxide

Chapter One: Introduction

1. Introduction

Most chemotherapy treatments cause disruption of spermatogenesis, resulting in a significant drop in sperm count and quality cells [Lopes et al., 2021]. Several different chemotherapy treatments cause different general side effects and defects in spermatogenesis [Poulopoulos et al., 2017]. In general, chemotherapies cause gonad toxicity due to the target rapidly proliferating cells [Allen et al.,2018]. Testicular injury with Leydig cells harm related to chemotherapy is one of the most general cytotoxicity consequences of treatment in male [Rahimi et al., 2022]. Male infertility is an extremely worrying problem, as a result, there is a growing need to address the issue and study preventive strategies [Kumar and Singh, 2015]. In addition, there are increasing numbers of males that was wished to have or want children after chemotherapy treatment [Okada and Fujisawa, 2019]. It was attributed to the improvements in cancer therapies, which the possibility of increasing survival.

Chemotherapies typically cause gonadotoxicity because they target actively proliferating cells [Howland, 2009]. The impact of chemotherapy on spermatogenesis is influenced by various factors, including the specific treatment, dosage, initial semen quality, and therapy schedule [Giwercman and Petersen, 2000]. Cytarabine (cytosine arabinoside, Ara-C) is a potent chemotherapeutic medication for treating various hematological malignancies such as acute leukemia and lymphomas [Doval et al., 2020; Wu et al., 2022]. It inhibits DNA synthesis and repair, leading to cytotoxicity in growing cells [Grant, 1997; Namoju et al., 2014]. Ara-C impairs spermatogenesis in humans and rodents in maturity.

Spermatogenesis is the progressive conversion of embryonic cells into spermatozoa that develops within the seminiferous tubular structures of the testis [James et al., 2020; and Wang et al., 2023]. It is dependent on a variety of hormones, including androgens are necessary for the proliferation and development of germ cells as well as somatic cells such as sertoli cells [Wistuba et al., 2023; Thumfart & Mansuy,

2023; Corpuz-Hilsabeck, & Culty, 2023]. Chemotherapy frequently leads to testicular injury and damage to Leydig cells, which are responsible for producing testosterone. This is a common side effect in men undergoing treatment [Holmes, S. J., Whitehouse, R. W., and Clark, S. T. (1994]. While many research findings have decreased the cytotoxic effects of numerous cancer medications, toxicity nevertheless persists as an issue requiring further enhancement. This study aims to demonstrate not only the impact of the cytarabine medicine on the fertility of male albino rats but also the protective effects of TTE following the administration of Ara-C chemotherapy.

Aims of the study

To investigate the protective effects of *Tribulus terrestris* on the rat testis following Ara C administration, and to determine the effectiveness of Ara C to produce a testicular toxicity via performing the following objectives:

1. Measuring of liver functions test (AST, ALT and ALP) in serum.
2. Assessment of oxidant (MDA) and antioxidant (CAT and SOD) Parameters in serum .
3. Measuring level of male hormones (Testosterone and 17 beta HSD FSH, and LH) in serum .
4. Semen Analysis (VCL, Motility%, Vigor, Integrity) in serum.
5. Apoptotic test by caspase 3 in serum .
6. Histopathological examination of the testis and epididymis.

Chapter Two: Review of the Related Literature

2. Review of the Related Literature

2.1. Chemotherapy Drug

Chemotherapy spreads via the bloodstream in the body. Thereby, it may process cancer cells almost anywhere in the body. Chemotherapy kills cells, which are in the process of splitting into two new cells (Sriharikrishnaa *et al.,* 2023). Body tissues are generated of billions of individual cells. When the cell is fully grown, most of the body's cells do not classify and multiply much, it only divides when they require to repair damage (Zhang *et al.,* 2013).

Consequently, chemotherapy is successful treatment methods in several malignances (Alam *et al.,* 2018); nevertheless, several problems are still related to chemotherapy as it utilizes a variant of greatly toxic agents (Anand *et al.,* 2024). The low specificity and great toxicity are possibly the problem with this kind of treatment (Zhong *et al.,* 2021). Chemotherapeutic agents have devastating side impacts; patients undergo chemotherapy generally die of general infections or other cancer and pneumonia (Da Silva and Casella, 2022).

The development of medical science is related to a paradigm shift in new anticancer therapies (Yeo *et al.,* 2018), resulting a significant increase in long life chance in patients (Zhang *et al.,* 2021). However, a tremendous enhancement has occurred in cancer chemotherapy, the serious adverse impacts related to the therapy are stayed a main challenge (Gavas *et al.,* 2021).

Treatment with anticancer drugs may have risky behavior, and the toxicity may produce to a deteriorated disorder life quality and survival time (Tannock *et al.,* 2022).

2.2. Cytarabine

Cytarabine (Ara C) is a chemotherapy agent and a nucleoside analogue utilized in the cancers treatment of white blood cells like AML and non-Hodgkin lymphoma and viral infections (Di Francia *et al.,* 2021). It involves teratogenic possible and result a variety of birth defects in fetuses and it kills cancer cells by inhibition of DNA polymerase through competition with deoxycytidine triphosphate, causing in the inhibition of DNA synthesis. It is known cytosine arabinose due to it which combines a cytosine base with an arabinose sugar (Ibrahim et *al.,* 2017).

It is an antimetabolic agent with the chemical formulawhich was the first discovered in the 1960s in Europe, and it was approved for use in the US in 1969 (Houshmand *et al.,* 2020), as shown in ***Chart (2.4)***.

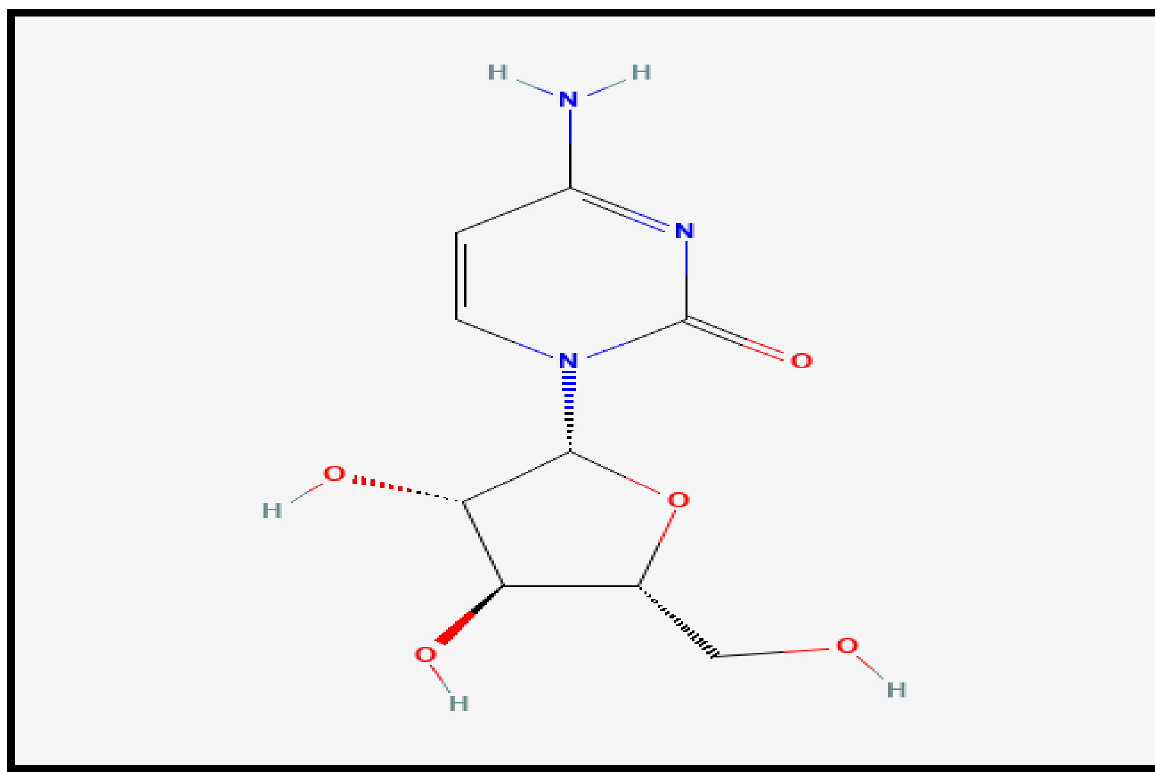


***Chart* 2.1:** The chemical formula of cytarabine (Arwanih *et al.,* 2022).

Cytosine normally combines with a different sugar, deoxyribose, to form deoxycytidine, a component of DNA (Rechkoblit *et al.,* 2019). Cytosine arabinoside is similar sufficiency to human cytosine deoxyribose (deoxycytidine) to be incorporated into human DNA, but different sufficiency that kills the cell. This mechanism is utilized to kill cancer cells (Zwueste *et al.,* 2023).

Cytarabine is utilized in standard stimulation regimens and maintenance therapy after remission (Majed, 2022). The phosphorylation of Ara C by deoxycytidine kinase produces Ara-C-triphosphate in cells. Ara C-triphosphate is combined into the duplicating DNA that triggers double-strand breaks via blocking DNA production. With the role of deoxycytidine kinase in the change of Ara C to the active shape, decreased mutation of deoxycytidine kinase in AML cells supports Ara C resistance in these cells (Chiou *et al.,* 2023).

Furthermore, a rise in oxidative phosphorylation is revealed in Ara C-resistant AML cells, although targeting mitochondrial metabolism to decrease oxidative phosphorylation in AML cells improves the anti-leukemic impacts of Ara C (Chen *et al.,* 2021). These results provide a promising modality to propose new therapeutic approaches for circumventing Ara C resistance in AML (Gurnari *et al.,* 2020). A great doses of Ara C have been recorded to produce side effects comprising cerebellar neurotoxicity, toxicity, leukopenia, anemia and thrombocytopenia (Han *et al.,* 2023).

2.2.1 Cytarabine's Cellular Metabolism and Pharmacokinetic

Ara C is controlled in the body via continuous intravenous (i.v.) infusion (Wu *et al.,* 2023). The drug is metabolized in the kidney liver, gastrointestinal mucosa, and granulocytes (Micozzi *et al.,* 2014). The treatment, when controlled

intravenously, has a primary distribution half-life of around ten minutes (Arwanih *et al.,* 2022).

Through the primary half-life, the common of the drug is metabolized in the kidney, liver and gastrointestinal tract into its inactive metabolite, uracil arabinoside (Majed, 2022). The secondary removal half-life is longer, and lasts one to three hours. The common of the dose controlled is excreted via the kidney within one day (Lombardo and Nichols, 2009). Ara C is also very toxic to the human body, and to mouse models (Park *et al.,* 2023). Some toxic effects of the drug are: thrombocytopenia, anemia, leucopenia, GI tract abnormalities, fever, pneumonitis and conjunctivitis (Raj *et al.,* 2021).

Ara C is an antineoplastic anti-metabolite utilized in the treatment of many forms of leukemia comprising acute myelogenous leukemia and meningeal leukemia (Alexander *et al.,* 2016). Anti-metabolites masquerade as purine or pyrimidine become the building blocks of DNA (El-Nashar *et al.,* 2023). They stop these substances becoming incorporated in to DNA through the "S" phase (of the cell cycle), stopping normal progress and division (Fajardo-Orduña *et al.,* 2021). Ara C is metabolized intracellularly into its active triphosphate form like cytosine arabinoside triphosphate (Nishi *et al.,* 2020). This metabolite that damages DNA by multiple mechanisms, comprising the inhibition of alpha-DNA polymerase, inhibition of DNA repair via an impact on beta-DNA polymerase, and incorporation into DNA (Yadav and Sawant, 2010).

2.2.2. Mechanism of Action of Cytarabine

Cytarabine (Ara C) arrives cells through nucleoside transport proteins, the most significant one being the equilibrative inhibitorsensitive (es) receptor (ABC) (Majed, 2022). When inside the cell, Ara-C needs activation for its cytotoxic impacts. The metabolism and mechanism of action of cytarabine are

directly connected to the biotransformation of its physiological deoxyribonucleotide counterpart, the normal nucleoside deoxycytidine, comprising membrane transportation, interaction with cellular targets and intracellular activation. Gene generates included in this procedure have been well featured and involve transporters of solute carriers (SLC) and ATP-binding cassette (ABC) families, activators like deoxycytidine kinase (DCK), nucleoside diphosphate kinase (NDK), and ribonucleotide reductase (Di Francia e*t al.,* 2021).

Intracellular Ara C is subsequently phosphorylated to Ara C monophosphate (Ara-CMP) via deoxy-cytidine kinase (dCK), after then to cytarabine diphosphate (Li *et al.,* 2017). Cytarabine triphosphate is a potent inhibitor of DNA polymerases that interferes with DNA chain elongation, DNA synthesis, and DNA repair (Di Francia *et al.,* 2021). Furthermore, Ara C is combined directly into DNA and functions as a DNA chain terminator that interfering with chain elongation (Tsuda *et al.,* 2020). Cytarabine catabolism includes two key enzymes, cytidine deaminase (CDA) and deoxycytidyne monophosphate deaminase (DCMD). These breakdown enzymes change cytarabine and cytarabine monophosphate to the inactive metabolites, uracil arabinoside and arabinosyluracil monophosphate. The other catabolic enzymes, which can impact cytarabine metabolism comprise pyrophosphatase and 5-nucleotidase. The balance between intracellular activation and degradation is a significant role in identifying the amount of drug, which is eventually converted to cytarabine triphosphate and, thereby, its subsequent cytotoxic andantitumor activity (Di Francia *et al.,* 2021).

Cytarabine is cytotoxic to a wide range of proliferating mammalian cells in culture (Drenberg *et al.,* 2016). It displays cell phase specificity, mainly killing

cells undergoing DNA synthesis (S-phase) and under specific disorders blocking the progression of cells from the G1 phase to the S-phase (Wang, 2022). While the mechanism of action is not wholly known, it observes that Ara C represent via the inhibition of DNA polymerase (Wang *et al.,* 2022). The latter mechanism is probably the most important. Cytotoxicity is highly specific for the S- phase of the cell cycle.

2.2.3. Serious Toxicities of Cytarabine

The selective procedure against quickly dividing cells and the decreased of metabolic activation in solid tumors have produced from broad activity, and concentrating the application of hematological malignancies (Saad, 2022).

The concentrated tolerable cumulated dose of Ara C is meaningfully lower when the agent is controlled as a continuous infusion, because of myelosuppression and gastrointestinal toxicity. On the contrary, continuous infusion can be less neurotoxic (Boddu *et al.,* 2023). The antileukaemic impact on continuous infusion high-dose Ara C is less well recognized (Konopleva *et al.,* 2022).

The only important toxicity of low-dose Ara C is myelosuppression. Specified the commonly poor disorder of leukaemia patients, low-dose Ara C therapy is well tolerated, though irregular cases of diarrhea (Zeidan *et al.,* 2022), reversible cerebellar symptoms, peritoneal and pericardial reactions, and ocular toxicity have been recorded (Mughal and Schrieber, 2010).

2.3. Infertility

Infertility is considered as unsuccessful pregnancy causing from regular and unprotected association between couples through a one year period (Akalewold *et al.,* 2022), and infertility occurrence among couples has been computed

around to be 15% (Safarinejad, 2008). Infertility etiology between couples includes 40%–50% of females only induced parameters (Mansour, 2023), 30% of males only induced parameters, and 20% of both males and females induced parameters (Khan *et al.,* 2023).

One of the objectives when assessing an infertile male rat is to determine reversible situations, which are responsible for infertility (Esteves *et al.,* 2011). Recurrent doses of exposed treatments can also cause infertility in males either directly by affecting the gonads or indirectly by affecting pituitary gonadotropins and causing changes in sperm parameters like, sperm count, morphology and mortality (Kesari *et al.,* 2018). Furthermore, there are several controversies around environmental parameters and occupational exposure to physical agents, which could impact fertility (Sakali *et al.,* 2023).

In the same context, chemical elements play a vital role in male reproduction due to defects of micronutrients or macronutrients can produce to spermatogenesis defects and reduced libido, causing in male rat infertility (Kumar and Singh, 2022).Different situations can cause infertility, direct or indirect males induced parameters compose about 30%–50% of infertility patients (Khan *et al.,* 2023). Congenital anomalies varicocele, urogenital infections, endocrine illnesses, and immunological parameters can be calculated among the etiologic parameters resulting infertility in male rat (Lundy and Vij, 2022). Radiation, weather and obesity and also impact fertility in male rat.

2.4. Spermatogenesis

Spermatogenesis is a complex process in which spermatogonial stem cells neighboring to the seminiferous tubule basal membrane of the testis undergo division (Houda *et al.,* 2021), to generate new population of stem cells and to

produce progeny cells, which change into spermatozoa immediately (Kubota and Brinster, 2018).

Spermatogenesis happens in all the cells in the seminiferous tubules of the testes undergo (Sertoli, 2018). It is the process whereby primitive, diploid, stem cell spermatogonia provide rise to greatly differentiated, haploid spermatozoa (Singh, 2023).

The process includes a sequence of mitotic divisions of the spermatogonia, the last process is given rise to the spermatocyte (Das *et al.,* 2023). Practically, the process of spermatogenesis is well conserved in all sexually proliferating organisms (Chocu *et al.,* 2012), although the size and shape of the mature sperm vary considerably among different species.

The spermatocyte is the cell that crosses the long process of meiosis starting with duplication of its deoxyribonucleic acid (DNA) through preleptotene (Saulnier *et al.,* 2022), coupling and condensing of the chromosomes through pachytene and at the end concluding in two reductive divisions to generate the haploid spermatid (Endo *et al.,* 2017).

The spermatid starts life as a simple round cell but quickly undergoes a sequence of complex morphological alterations (Miyaso *et al.,* 2022). The nuclear DNA converts greatly condensed and elongated into a head region that is covered by a glycoprotein acrosome coat while the cytoplasm develops a whip-like tail enclosing a flagellum and tightly-packed mitochondria (Sharma and Agarwal, 2018). The progressive morphological stages in the differentiation of the spermatid (12 stages of spermiogenesis) provide the base for the determination of the stages of the spermatogenic cycle in the male rat (Wang *et al.,* 2018). The first division produces secondary spermatocytes (SS) and the second division produces the haploid spermatid. This is followed by the

transformation of round spermatids into elongated (spermiogenesis) (Drevet *et al.,* 2022), as shown in ***Chart (2.3)***.

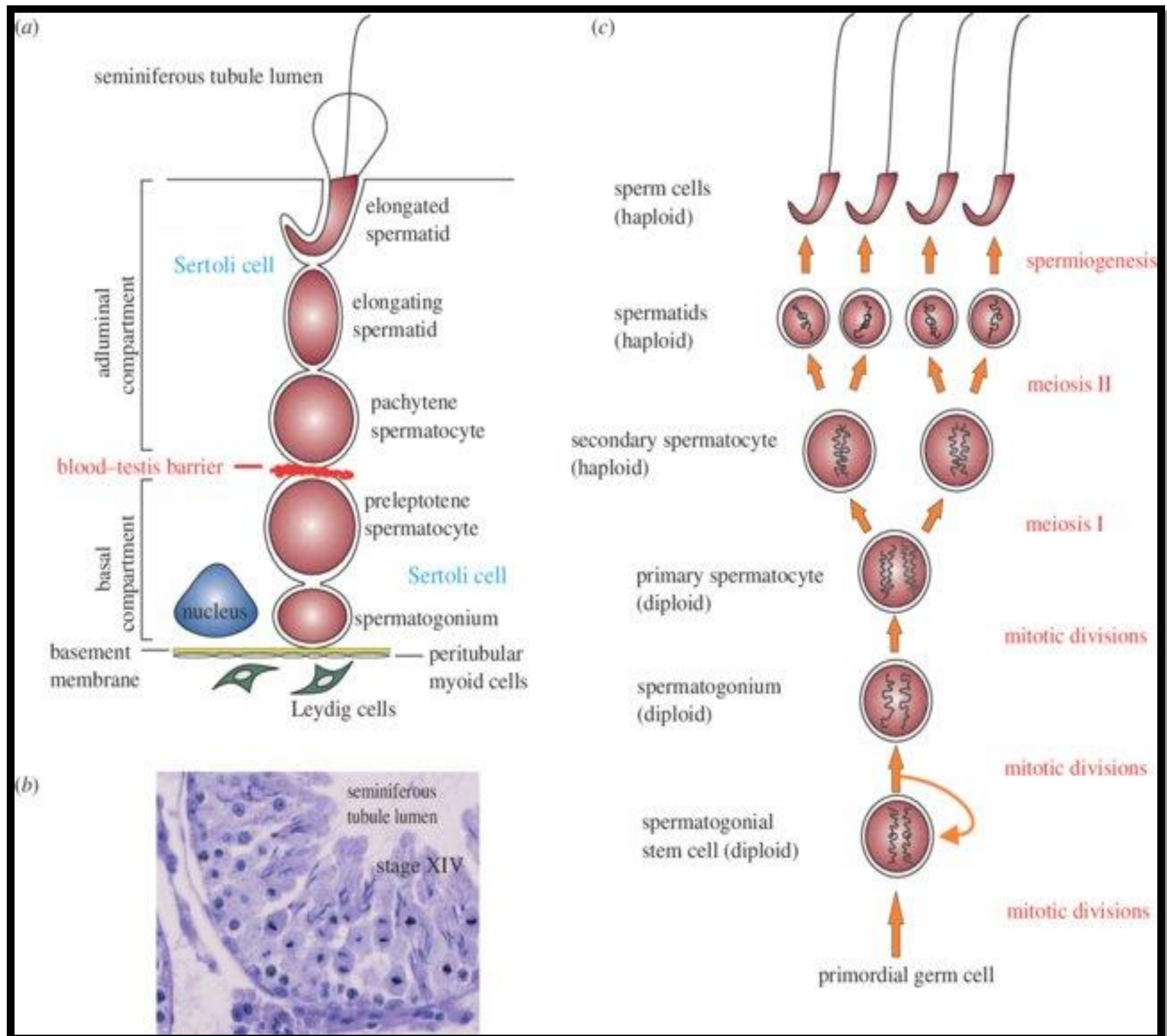


Chart 2.2: The stages of spermiogenesis (Cheng and Mruk, 2010).

The highly condensed and mature spermatozoa are then released into the seminiferous tubule lumen (Fang *et al.,* 2022). Both spermatids are expressed activator CAMP-responsive element modulators (CREMs) that are reflected meiotic stage markers, whereas the meiotic and post-meiotic stages comprise elongated spermatid and spermatozoa-expressed ACROSIN (Khaleel *et al.,* 2022).

Moreover, it is a significant role for cancer treatment, chemotherapy may rise the survival time of cancer patients (Wang *et al.,* 2022). Nevertheless, chemotherapy also has several side effects, which can affect the life quality after taking treatment (Li *et al.,* 2022). The impact on fertility is a subject of huge concern, particularly for male fertility (Skakkebaek *et al.,* 2022).

Reproductive side effects on male individuals comprise the damage to the testicular germinal epithelium and the sperm microenvironment, and the condition of spermatogenesis and sperm maturation (Nikmahzar *et al.,* 2023).

2.4.1. Parameters inducing spermatogenesis impairment and male infertility

Spermatogenesis is impacted by a myriad of parameters, which are shared in the development of subfertility (Chao *et al.,* 2023). Sperm dysfunction is the most general produce of infertility in male. Male infertility is observed on the basis of the existence of “oligozoospermia” via reduced sperm count, “abnormal sperm” via abnormal morphology and “asthenospermia” via reduced sperm motility (Chao *et al.,* 2023). Oxidative stress produces sperm dysfunction. The disorder associated with the balance between oxidants and antioxidants can have a strong toxic impact on spermatogenesis via the generation of excessive oxidants and male infertility (Habib *et al.,* 2019).

Moreover, it causes from raised lipid peroxidation influenced by reactive oxygen species and the consequent antioxidant depression that was revealed in these individuals (Barbosa *et al.,* 2020).

Huge concentrations of iron and cadmium augmented oxidative stress in the sperm homogenate and seminal plasma of the infertile set, reduced the content of antioxidants, and thereby had a strong toxic impact on spermatogenesis by

generating excessive oxidants and influencing apoptosis (Habib *et al.,* 2019). Different genetic abnormalities have been related to impaired spermatogenesis (Katami *et al.,* 2023).

2.5. Reproductive Hormones.

Two anterior pituitary hormones follicle stimulating hormone and luteinizing hormone are included in spermatogenesis and testosterone creation (Ayman *et al.,* 2021). Gonadotrophins (LH), (FSH) and testosterone are the main components of germ cell growth (Ramaswamy and Weinbauer, 2014).

The positive and complete male germ cell growth relies on the balanced endocrine relationship of hypothalamus, the testis and pituitary (Ilacqua *et al.,* 2018).

Episodic secretion of Gonadotrophin revealing Hormone (GnRh) via the hypothalamus elicits the pulsatile reveal of gonadotrophins FSH and LH. Follicle stimulating hormone and luteinizing hormone are the significant components of steroidogenesis in the gonads (Abreu and Kaiser, 2022).

Luteinizing hormone is a private steroidogenic hormone performing only on the interstitial cells of Leydig, while FSH performs completely on the Sertoli cells, which are existed in the testes (Anso, 2023).

Follicle stimulating hormone links with receptors in the Sertoli cells and motivate spermatogenesis (De Pascali *et al.,* 2018). Luteinizing hormone motivates the creation of testosterone in Leydig cells. Testosterone, in turn relies on the Sertoli cells and peritubular cells of the seminiferous tubules and motivates spermatogenesis (Talapatra, 2021).

Moreover, testosterone, estradiol and inhibin regulator the secretion of gonadotropins. Testosterone, the main secretory produce of the testes, is the chief inhibitor of LH secretion in males (Sengupta *et al.,* 2019). It is metabolized in peripheral tissue to the powerful androgen, dihydrotestosterone or the powerful estrogen, estradiol (Alemany, 2022).

T.Terrestre's has been considered as a potential stimulator of testosterone production, which has been related with steroidal saponins prevailing in this plant (Pavin et al., 2018).

Cyclophosphamide (CP) is the most commonly used anticancer and immunosuppressant drug, which causes several toxic effects, especially on the reproductive system. Patients who need to use CP therapy exhibit reduced fertility or infertility, which impacts both physically and emotionally on the decision to use this drug, especially among young males. They hypothesized that the treatment with Tt dry extract would protect the male reproductive system against CP toxicity (Pavin et al., 2018b).

2.6. 17 Beta-Hydroxysteroid dehydrogenases

Hydroxysteroid dehydrogenases (HSD) include a major family of enzymes, which are comprised in the biogenesis and metabolism of several steroid and non-steroid substrates in both animals and human. Steroid hormones work through specific receptors that activate gene transcription. These are nicotinamide adenine dinucleotide (NAD) (phosphate) with ((P)/NAD(P)H)-dependent oxidoreductases that interconvert ketones and the matching to secondary alcohols at changed locations of steroidal substrates (3α-, 3β-, 11β-, 17β-, 20α- and 20β-location).

Specified their characters in steroid metabolism, HSD family members play significant biological characters in human health and have been related to different diseases. For example, members of the 17beta-HSD subfamily have been related to breast and prostate cancer, polycystic kidney cancer, and Alzheimer's disease, to term a few.

Though different 17β-hydroxysteroid dehydrogenases are generally called "isozymes" this could be avoided as the 17β-HSDs are implied by different not homologous genes with recognized amino acid (AA) sequences releasing different subcellular localizations, and cofactor and substrate favorites.

17β-HSD belongs to two protein superfamilies: short chain dehydrogenase/reductase (SDR) and aldo-ketoreductase (AKR).

17β-hydroxysteroid dehydrogenases facilitat the decreased of estrone to estradiol, dehydroepiandrosterone to androstendiol and dihydrotestosterone to 3α- and 3β-diol and 17β-HSD arbitrates the oxidation of estradiol to estrone, testosterone to androstenedione and androstendiol to dehydroepiandrosterone.

2.7. Impact Chemotherapy on Infertility

Chemotherapies cause gonadotoxicity due to the target quickly proliferating cells (Yahya *et al.,* 2022). The risk impact of chemotherapies on spermatogenesis relies on multiple factors, like the kind of treatment, dosage, initial semen quality, and the regimen of therapy (Eugeni *et al.,* 2022).

The increased population of cancer disease survivors and is the attached by an increased risk of unwanted infertility (Gilleland Marchak *et al.,* 2018). These reasons have shifted researchers' interest in understanding treatment-induced sequelae, specially the impacts of cancer and/or treatment on fertility (Rodriguez-Wallberg *et al.,* 2023).

The cells save on dividing till there are cells mass in cancer. These cells mass develops a swelling, known a tumor (Esmaeilzadeh and Nasirzadeh, 2022). Some treatments kill dividing cells by damaging the portion of the cell's control center, which allows it divides, the other treatments interrupt the chemical processes related to cell division (Xu *et al.*, 2022).

The division of chemotherapy damages cells as they divide, nucleus is a dark spot in the control center of each living cell (Cremer *et al.,* 2020), it contains chromosomes that are made up of genes, these genes must be copied exactly each time a cell divides into two in order to create new cells, ***Chart (2.1).***

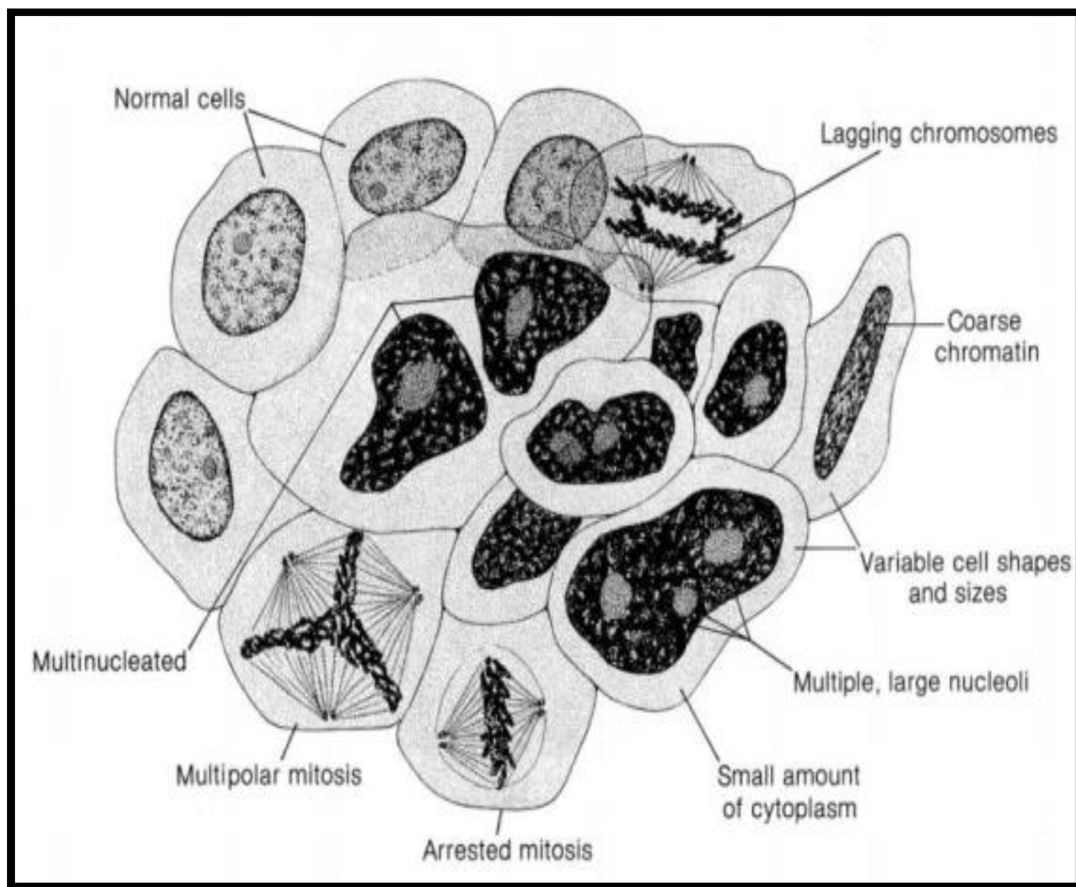


***Chart* 2.3:** The comparison between normal and cancer cell (Grewal, 2014).

Chemotherapy treatment damages the genes inside the cell's nucleus, some treatments damage cells at the splitting point (Kiss *et al.,* 2021). Moreover, some damage the cells while the cells are creating copies of all their genes before splitting process (Duy *et al.,* 2021).

Chemotherapy is much less possibly to damage cells that are at rest, like the most normal cells. It must be combined of different chemotherapy treatment (Salas-Benito *et al.,* 2021). This will comprise treatments, which damage cells at different phases in the cell division process. This implies there's more chance of killing more cells (Saini *et al.,* 2020).

2.8. Medical herb and it is uses in Fertility Management.

The previous studies of indigenous systems of medicine has indicated that plant preparations have a to in fertility management (Khadim *et al.,* 2023). Currently, researchers became interested in the function of these native plant compounds in the induction of male and female fertility. (Okwute *et al.,* 2023).

The usage of medicinal plants is on the rise as an outcome of the move away of attention from synthetic treatments to old-fashioned medical (Sigolo, 2022). The majority of old-fashioned treatments are dependent on herbs, which are used around 80% of the global population (Bhakta and Das, 2020).

Some uses of medicinal plants are like improving reproductive functions. while other herbal remedies are utilized to treat different reproductive cases, natural aphrodisiacs are consumed to improve sexual performance, like oligospermia, erectile dysfunction, hormonal imbalance, azoospermia, etc. (Ramgir *et al.,* 2021). Consequently, it is necessary to study larger scale, randomized clinical trials to display the role of traditional herbs in male infertility management (Bouabdallah *et al.,* 2024).

There still occur to be several more preparations of medicinal plants, which have not been featured to identify the phytochemicals unique to them, which have target special effects on the gonads. Additional, cooperative efforts at isolating pro-drug candidates from medicinal plants for studies at the molecular, cellular and clinical level towards elucidating their characteristics of action on the testes are thus reasonable in the light of the present male fertility crisis (Abarikwu *et al.,* 2020).

2.9. *Tribulus terrestris* Herb

Tribulus terrestris (Tt) is defined as a pubescent herb of the Zygophyllaceae family and is reflected a weed, which grows generally in tropical and moderate climate regions of the world (Singh *et al.,* 2023). The term of "*Tribulus*" in Greek is a diuretic (Neumann *et al.,* 2024).

Tribulus is used in different countries like Vietnam and China for the medication of Epitasis, postpartum haemorrhage, and gastrointestinal bleeding. It is also used in South Africa remedy for arthritis and inflammation. Among its attributes, the possibility to rise serum testosterone levels stands out. Thereby, it has been used in the treatment of infertility and erectile dysfunction. In Europe, it is used as the medication for impotence and as stimulants to aid enhance sexual desired and performance. *Tribulus* is raised sperm production and testosterone level in male (Akbar and Akbar, 2020).

While in female, it was observed that the increase in the hormones concentration with testosterone in order to become so slightly influenced,

thereby enhancing reproductive function, ovulate and libido (Liu *et al.*, 2021), the shape of *T. terrestris* herb is shown in ***Chart (2.5)***.

***Chart* 2.4:** *Tribulus terrestris* herb.

Tribulus involves three groups of active prodigiosin, diosgenin and photochemical discoing (Goswami *et al.*, 2021). These substances have an impact on sexual performance and can treat different sexual disorders. They adjust sexual energy level and strength by rising the percentage of free testosterone levels for male and they impact progenolone, progesterone and oestrone (Shawish *et al.*, 2021). *Tribulus* was observed to be rich source of calcium (Tkachenko *et al.*, 2020).

The effect of *Tribulus Terrestre's* extract immunohistochemical and pathophysiological against induced toxicity of cytarabine on reproductive system in male rats, and this impact seems to be refereed by its antioxidant action and it also stimulates the production of testosterone from the Leydig cell (Pavin *et al.*, 2018).

2.9.1. The advantage of *Tribulus terrestris* for fertility

T. terrestris herb is considered as one of several herbs that have been observed to benefit for improving fertility and boost male test (Jaradat and Zaid, 2019). *Tribulus* has been utilized as a classical fertility herb for years ago, in both classical Chinese medicine and Ayurveda. Classical healing practices are stemmed in time tested findings, but scientific studies in Bulgaria desired to understand how this herb really mechanisms (Jarić *et al.*, 2018).

Many research have been studied to find out how *Tribulus* helps fertility for male (Khaleghi *et al.*, 2017; and Santos *et al.*, 2019).

The advantage of *Tribulus terrestris* herb for fertility can be summarized by (Alahmadi, 2020) as follows: (1) rise in sex hormone generation in male; (2) rise in serum follicle stimulating hormone; (3) rise in luteinizing hormone and testosterone in male; (4) enhancement in sexual desire in male; (5) complete fertility tonic for the male reproductive; (6) rise in sperm count, motility, and normal morphology; (7) reduction in the impacts of antisperm antibodies; (8) can help the male body in generating DHEA to treat erectile dysfunction, and (9) defensive to liver health; increases glutathione (strong antioxidant) levels that are significant to cellular health and immunity.

2.9.2. Erectile Dysfunction

The chief part of Tribulus, which helps in fertility for male, is a constituent commonly known protodioscin (Azam *et al.*, 2019). This constituent helps to enhance DHEA levels in the male body (Malviya *et al.*, 2016). It has observed that levels of DHEA are low when erectile dysfunction existed for male (El-Sakka, 2018).

Some studies have observed that protodioscin, produced from *Tribulus*, rises natural DHEA levels required for positive erection. Protodioscin is also the chief constituent, which is responsible for Tribulus' aphrodisiac qualities. A rise in sexual is suitable when utilizing this plant type for male rat (Ştefănescu *et al.,* 2021).

2.9.3. Antisperm Antibodies

Antisperm antibodies are the findings of the body having an immune response to semen and a 'standard procedure' indicating the event of sperm agglutination (Silva *et al.,* 2021).

This can occur not only in male, but it is also in female. Antibodies that are triggered through the immune response effort to kill the sperm due to the body recognizes them as foreign invaders (Kutteh *et al.,* 2019).

Furthermore, Chinese classical medicine and Ayurvedic practitioners have observed Tribulus to be very active in enhancing sperm count, motility, and normal morphology when mixed with dietary and exercise changes (Inwati *et al.,* 2022).

Chapter Three: Methodology

3. Methodology

3.1. Material

3.1.1. Equipment and instruments

Equipment and instruments used in this study was shown in table (3.1).

Table 3.1: The equipment and instruments their manufacturer and country sources

NO.	Equipment & Instruments	Company	Country
1.	Analytical sensitive balance	Sartorius	Germany
2.	Balance for animals	Shimadu company	Japan
3.	Centrifuge	Hettich Roto fix11	Japan
4.	Digital camera	Toup Cam	China
5.	Electronic Balance	Metter company	Switzerland
6.	ELISA reader	Biotic	USA
7.	ELISA washer	Biotic	USA
8.	Freezer	Hitachi	Japan
9.	Incubator	Binder	Germany
10.	Jell tube	AFMA-Dispo	Japan

11.	**Latex gloves**	**Great glove**	**Malaysia**
12.	**Light microscope**	**Leica**	**China**
13.	**Optical microscope with table PC**	**OPTICA**	**Italy**
14.	**Rotary macro tom**	**Leica**	**China**
15.	**Spectrophotometer**	**Labomed**	**UK**
16.	**Sterile syringes 5, 10 ml**	**Proton**	**Malaysia**
17.	**Water bath**	**K.F.T.Lab. Equipment**	**Italy**
18.	**Plan tube**	**AFMA-Dispo**	**Japan**

3.1.2. Chemicals

Table (3.2) outlines the sources of all the chemical supplies, purified reagents, and standard kits used in the current study.

Table 3.2: Chemicals kits

No.	Chemicals	company	Country
1.	ALT Kit	Agappe diagnostic	India
2.	AST Kit	Agappe diagnostic	India

3.	**ALP Kit**	**Agappe diagnostic**	**India**
4.	**Caspase kit**	**Laboratorio ct**	**Italy**
5.	**SOD kit**	**Laboratorio ct**	**Italy**
6.	**MDA kit**	**Laboratorio ct**	**Italy**
7.	**Testosterone Kit**	**Monobind Inc**	**USA**
8.	**Luteinizing Hormone LH**	**Monobind Inc**	**USA**
9.	**Follicle Stimulation Hormone FSH**	**Monobind Inc**	**USA**
10.	**17 Beta - HSD**	**Monobind Inc**	**USA**
11.	**Caspase elisa kit**	**ELK Biotechnology**	**China**
12.	**Cytarabine drug (chemotherapy)**	**SOLARBIO**	**China**
13.	**Normal saline**	**Labort**	**India**
14.	**Paraffin Wax**	**Merck**	**Germany**
15.	**Ethanol**	**Merck**	**Germany**
16.	**Formalin 10 %**	**TEDIA Company**	**USA**

3.2. Methods

3.2.1. Preparation of Drug Cytarabine

Cytarabine, also referred to as Ara-C, is a chemotherapeutic drug that is mostly used to treat hematological malignancies, including acute myeloid leukemia and non-Hodgkin lymphoma. Cytosar-U® was injected intraperitoneally (Benkessou, 2019). The active component is a white, crystalline powder with the molecular weight of 243.22 g/mole and the chemical formula $C_9H_{13}N_3O_5$. First, 10 milliliters of distilled water were used to dissolve 25 mg/kg of cytarabine. This cytarabine solution was made right before usage (Namoju & Chilaka 2021).

3.2.2. Preparation *Tribulus terrestris*

The following procedure was used to manufacture the extract: The fruits of *Tribulus terrestris* were harvested from their natural habitats in Iraq, and the extraction process involved several steps. Plant samples were air dried, rinsed with water, and then dried. Following the water's evaporation, the leftovers were 250 g of powdered extracted using 500 milliliters of 70% ethanol in a soxhlet system, and the extracts were dried using a rotary evaporator set at 65 degrees Celsius, according to (Hussain *et al.,* 2009).

3.2.1. Quantification of Individual Phenolic Compounds Using HPLC Analysis of *Tribulus terrestris*

Quantification of individual phenolic compounds was performed by reversed phase HPLC analysis, using a SYKAM HPLC chromatographic system equipped with a UV detector). An effective technique that is often used due to its sensitivity and simplicity, this approach has multiple advantages (Gałęzowska *et al.,* 2021). The column was -C18-OSD (25cm, 4.6mm). The column temperature was 30ºC the gradient elution method, with eluent A (methanol) and eluent B (1% formic acid in water (v/v)) was

performed, as follows: initial 0-4 min, 40 % B; 4-10 min, 50 % B; and flow-rate of 1.0 mL/min. The injected volume of samples 100 µL .and standards was 100 µL and it was done automatically using autosampler. The spectra were acquired in the 280 nm.

3.3. Experimental Animals

The experiment was carried at the laboratory animal's facilities college of Veterinary Medicine College / University of Kerbala. Twenty- rats (albino rats) were used in this study with an average Wight between 280-300 g. These animals were kept in suitable environmental condition with a temperature of around 25-28 C°, relative humility 40 ٪- 60 ٪conditions room with a 12:12 h/light/dark cycle (Meyer *et al.,* 1982). The animals were housed in aplastic cage with diameters of 50×35×15 cm. The food given was pellets. The animals were kept for at last 15 days for acclimatization before experiment began.

3.4. Experimental Design

Twenty adult male rats were randomly divided into four groups comprising five animals for each group as the following:

* First group: (G1): Five male rats were injected normal saline (0.1 ml, daily, intraperitoneally injection) and served as the control.

* Second group: (G2): Five male rats were injected single concentration of Cytarabine (25 mg/kg/BW) intraperitoneally injection (Namoju and Chilaka, 2021).

* Third group: (G3): Five male rats were *Tribulus terrestris* (250 mg/kg/BW) orally for 4 weeks (Ojha-Nandave *et al.,* 2020).

* Fourth group: (G4): Five male rats were injected a concentration of Cytarabine (25 mg/kg/BW) intraperitoneally and *Tribulus terrestris* (250 mg/kg/BW) orally for 4 weeks. (Namoju and Chilaka, 2021).

3.5. Ethical Approval

All experimental technique was authorized by the College of Veterinary Medicine of Kerbala and complied with the ethical approval number (UOK.VET. PH.2023.078).

3.6. Preparation and Collection of the Samples

At the end of the experiment, the animals were anesthetized by chloroform inhalation, the chest and abdomen were open by thoracotomy and laparotomy. followed by collection of blood to perform biochemical and take the testes for histopathological study.

Rats used in experiments are put in a covered jar with cotton that has been rinsed with chloroform to make them unconscious. They are then put through a cardiac puncture using sterile syringes, where a needle is inserted into the heart to carefully extract 5 milliliters of blood. The blood sample is placed in a gel tube without anticoagulant, and it is left for about 30 minutes at room temperature to properly agglutinate. After that, the serum would be separated in an eppendorf tube using a centrifuge set at 3000 rpm for fifteen minutes, and it would be stored frozen at -20 °C.

3.7. Biochemical Parameters

3.7.1. Liver Enzymes

3.7.1.1. Serum Aspartate Aminotransferase Determination

The aspartate aminotransferase (AST) activity in the serum is measured using an aspartate aminotransferase test kit (Agappe diagnostic, India code 683-562) (Bergmeyer *et al.,* 1976; and Thefeld, *et al.,* 1974), as shown in Appendix (I).

3.7.1.2. Serum Alanine Aminotransferase Determination

Serum levels are measured using an Alanine Aminotransferase Test Kit (Agappe Diagnostic, India, Clin. Chem, Thefeld *et al.,* 1974), as shown in appendix (II).

3.7.1.3. Serum Alkaline Phosphatase Determination

Serum alkaline phosphatase levels are measured using an alkaline phosphatase test kit (Agappe diagnostic, India) according to appendix VI's illustrations in (Schlebusch *et al.,* 1974), as shown in appendix (III).

3.7.2. Oxidant Enzyme

3.7.2.1. Serum Malondialdehyde Measurement:

This approach of oxidative stress assessment is necessary to evaluate lipid peroxidation properly in disease situations. Lipid peroxidation naturally produces MDA and 4-hydroxynonenal (4-HNE). A widely accepted technique for evaluating oxidative damage is to quantify the products of lipid peroxidation. The MDA Microplate Assay Kit makes it simple to find MDA in a variety of samples. The MDA-TBA adduct is created in the sample when thiobarbituric acid (TBA) combines with

MDA. The MDA-TBA adduct is easily measured with a colorimeter (λ= 532 nm). As stated by Kavsak *et al.* (2017) and shown in appendix (IV).

3.7.3. Antioxidant Enzymes

3.7.3.1. Catalase Activity

The catalase (CAT) activity was measured using spectrophotometry, following the procedure described by Aebi, (1984). This approach involves tracking the decrease in H2O2 concentration at 240 nm in the presence of a 20 µL sample (S1). Enzyme activity is quantified in units, where 1 unit corresponds to the decomposition of 1 micromole of H2O2 per minute at pH 7 and a temperature of 25 degrees Celsius, within Appendix (V).

3.7.3.2. Superoxide Dismutase Activity.

The process of converting superoxide radicals (O2-) into hydrogen peroxide (H2O2) and elemental oxygen (O2) is facilitated by superoxide dismutases (SODs). These enzymes play a vital role in protecting against the harmful effects of superoxide radicals. Tumor cells in mice lacking SOD1 and SOD2 are resistant to apoptosis, although these animals develop liver cancer spontaneously. NBT is enzymatically transformed to NBT-diformazan through the action of xanthine oxidase (XOD) and hydrogen peroxide in the Superoxide Dismutase Microplate Assay Kit. NBT-diformazan absorbs light with a wavelength of 560 nm or more. Superoxide scavengers (SOD) decrease the concentration of superoxide ions, resulting in a decrease in the rate of formation of NBT-diformazan. The decrease in NBT-diformazan is a reliable indication of SOD activity in experimental samples, as stated by Kavsak *et al.* (2017) within Appendix (VI).

3.8. Sex Hormones Assay

Analyze hormone levels using the Enzyme-Linked Immunosorbent Assay (ELISA) method. Specifically, measure the levels of Testosterone, Follicular Stimulating Hormone (FSH), and Luteinizing Hormone (LH). Enzyme-linked Immunosorbent rely on the employment of an enzyme to identify the interaction between antigens and antibodies. The enzyme produces a brightly colored end product when it binds to Ag: Ab, using a substrate that has no color (Ma and Shieh, 2006).

3.8.1. Estimation of Testosterone Hormone Concentration

The concentration of serum testosterone hormone (T) in the current investigation was determined using the ELISA technique, employing a commercially available test kit as specified in Table 3-2. This study employs a biotin double antibody sandwich technology-based enzyme-linked Immunosorbent test (ELISA) to quantitatively determine the amounts of Testosterones in the samples (Mujika *et al.,* 1996). The testing technique was conducted in accordance with the manufacturer's instructions, as outlined in Appendix (VII).

3.8.2. Estimation of 17β-Hydroxysteroid Dehydrogenase Activity.

The activity of 17β-HSD was measured following the protocol described by (Jarabak *et al.* 1962). The 200 µL of supernatant fluid was combined with 950 µL of a sodium pyrophosphate buffer (pH 8.9) at a concentration of 440 µM. Additionally, 250 µL of bovine serum albumin (25 mg crystalline BSA) and 20 µL of 0.3 mM 17 β-estradiol were added to the mixture. The enzymatic activity was quantified as the amount of nanomoles of NADH produced per minute per milligram of protein.

3.8.3. Estimation Follicular Stimulating Hormone Concentration :

The concentration of serum follicle stimulating hormone in the current investigation was determined using the ELISA technique, utilizing a commercially available test kit as specified in Table 3-2. The FSH levels in samples were measured using an enzyme-linked immunosorbent assay (ELISA) that utilizes a biotin double antibody sandwich technology (Di-Simoni *et al.,* 1997). The testing procedure followed the manufacturer's instructions, as shown in appendix (VIII).

3.8.4. Estimation of Luteinizing Hormone Concentration:

The current study assessed the concentration of Serum Luteinizing hormone using the ELISA technique and a commercial test kit provided in Table 3-2. The LH levels in samples were measured using an enzyme-linked immunosorbent assay (ELISA) that utilizes a biotin double antibody sandwich technology. The testing procedure followed the manufacturer's instructions, as outlined in appendix (IX), (Uotila *et al.,* 1981).

3.9. Semen Analyses

3.9.1. Sperm Motility and Vigor

To assess the movement and strength of the semen, it was mixed with 50 µL of DMPBS (Nutricell) and examined subjectively using a phase-contrast microscope at 100x magnification. Additionally, an objective analysis was conducted using the semi-computerized system Sperm Class Analyzer (SCA) to measure the curvilinear velocity (VCL—µm/s). The evaluations were conducted by the same examiner.

3.9.2. Membrane Integrity

The assessment of membrane integrity was conducted using a combination of two probes, propidium iodide (PI) and carboxyfluorescein diacetate, following the method outlined by (Harrison and Stringer, 1990). Following the incubation period, a total of 200 spermatozoa was observed under a magnification of 400x using epifluorescence illumination. Spermatozoa that exhibited complete fluorescence over their whole length following staining with carboxyfluorescein diacetate were categorized as intact, whilst all other spermatozoa were classed as damaged.

3.10. Assessment the Activity of Caspase 3 (Apoptotic Test)

Experiments were conducted to assess the activity of serum caspase enzyme, for this purpose ELISA kit were used. The plate has been previously coated with Rat CASP3 antibody. The CASP3 protein, which is present in the sample, is introduced and forms a bond with the antibodies that are attached to the wells. Subsequently, the Rat CASP3 Antibody that has been biotinylated is introduced and forms a binding interaction with CASP3 present in the sample. Next, Streptavidin-HRP is introduced and forms a strong connection with the Biotinylated CASP3 antibody. Following incubation, all unbound Streptavidin-HRP is removed using a washing process. Next, the substrate solution is introduced, and the color intensity increases in direct correlation with the concentration of Rat CASP3. The reaction is halted by adding an acidic stop solution, and the absorbance is quantified at a wavelength of 450 nm.

3.11. Histopathological Study of Testis and Epididymis.

After a period of 28 days, all of the animals underwent a deep chloroform anesthetic, were euthanized, and had their testes and epididymis surgically extracted from the surrounding connective tissue and fat. After a brief rinsing with tap water, they were promptly submerged in a 10% formal saline solution. The histological changes of each animal's organs from one side were studied according to Mescher method, (2010). The sections were stained using standard hematoxylin and eosin (H&E) staining methods. To analyze the sections being examined, microscopy images of each section were captured using a light microscope and a digital imaging device from Canon, Japan. Within Appendix (X).

3.12. Data Analysis

The data were analyzed using Graph Pad Prism version 7.0 for Windows (Graph Pad Software, San Diego, USA) and are presented as the mean ± standard error of the mean. Before doing comparative statistical analysis, normality tests were performed using the D'Agostino and Pearson normality test. while analysis of variance (ANOVA) with Tukey's multiple comparisons test was utilized to analyze differences among more than two groups. A difference was considered statistically significant at a significance level of $P < 0.05$.

Chapter Four: Results and Discussion

4. Results and Discussion

4.1. Quantification of Individual Phenolic Compounds Using HPLC Analysis of *T. terrestris*

Table 4.1: Quantification of individual phenolic compounds using HPLC analysis of *T. terrestris*

Name	Con. (ppm)
Gallic acid	**20.6**
Apigeniun	**18.9**
Louteolin	**12.6**
Caffeic acid	**16.9**
Ferulic acid	**20.4**
p-coumaric acid	**13.6**

High-performance liquid chromatography

Phenolic compounds such as quercetin, apigenin, and luteolin have been reported to boost Leydig cell activity, enhancing testosterone production by upregulating steroidogenic proteins(Ye, R. J. et al 2020). Furthermore, catechin has been found to potentiate the stimulatory effects of gonadotropin-releasing hormone on LH release and human chorionic gonadotropin on testosterone release by Leydig cells in vitro(Yu, P. L.,et al 2010).

4.2. Biochemical Parameters:

4.2.1. Assessment the Activity of Liver Function Enzymes

Experiments were conducted to assess the level of serum liver enzymes following administration of TTE and Ara C in male rats.

Post-hoc analysis using Tukey's multiple comparisons test indicated that animals treated with Ara C were significantly increased in all examined liver function enzymes i.e. AST level (195±3.37), ALT level (128.2±1.96), ALP level (130.2±1.89) as compared to control group AST level (146.3±3.37), ALT level (72.91±1.96), ALP level (80.92±1.89) ***Chart*** (***4.1***). In addition, male rats treated with TTE have shown no significant effect (AST: P= 0.78; ALT: P=0.48; ALP: P=0.57) as compared to control group.

Interestingly, AST, ALT, and ALP levels were significantly reduced ($P<0.0001$) following administration of the TTE + Ara C (AST: 158.7±3.37; ALT: 81.81±3.37; ALP: 87.25±1.89) in comparison with Ara C treated group (AST: 195±3.37; ALT: 128.2±1.96; ALP: 130.2±1.89) ***Chart*** (***4.1***).

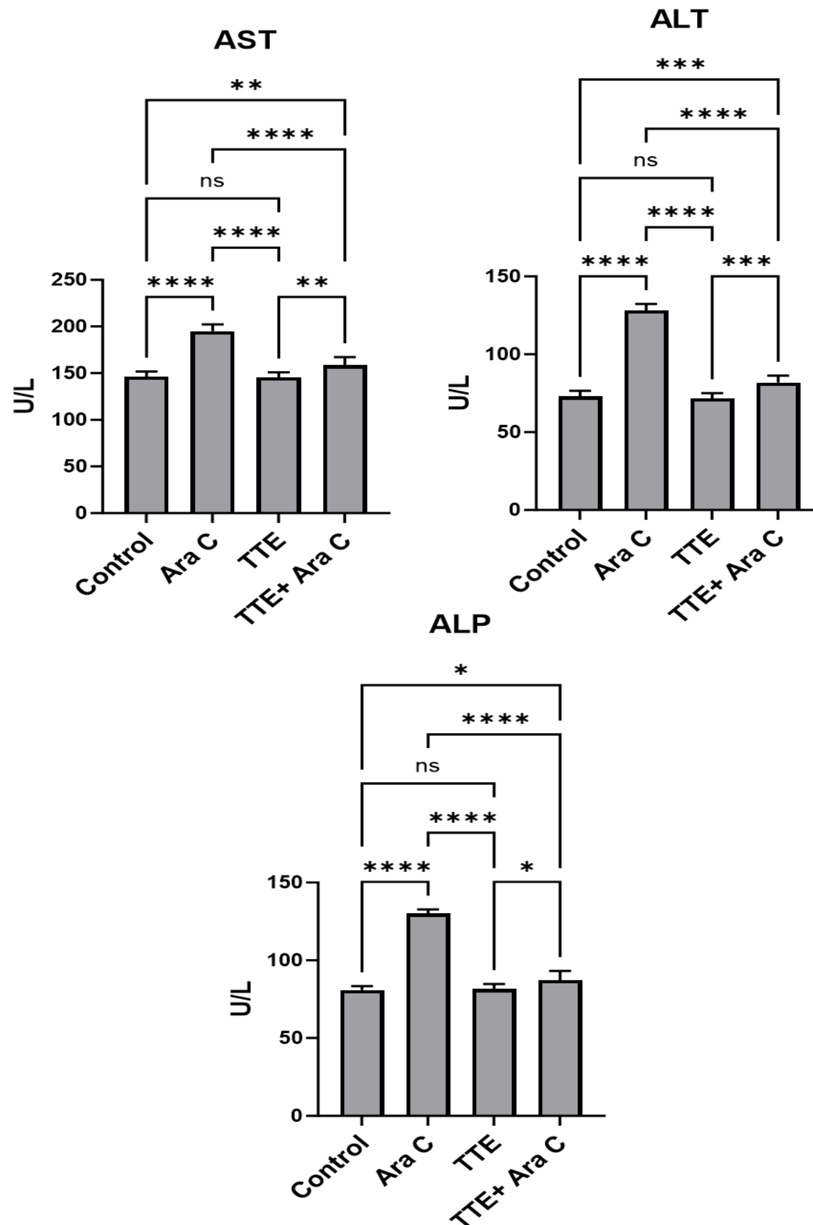


***Chart* 4.1: Effect of TEE and Ara C on the serum liver enzymes in male rats**

Effect of TTE and Ara C on serum liver enzymes in male rats for 4 weeks. Treated animals with 25 mg/kg. BW IP of Ara C showed a significant increase (****P < 0.0001) in AST, ALT, and ALP compared to control negative group (treated with normal saline). Male rats treated orally with 250mg/kg. BW of TTE have shown no significant effect in all liver enzymes compared to control negative group. A combination of TEE+Ara C significantly decreased (****P < 0.0001 in AST, ALT, and ALP) in comparison with control positive group. *: values significantly different (P= 0.01). **: values significantly different (P= 0.003). Data are expressed as mean ± SEM, n = 5.

The aim of this study was to assess the protective and antioxidant effects of TTE against testicular toxicity caused by Ara C in rats. Additionally, the study aimed to investigate the potential mechanisms of protection provided by TTE. In addition, the effectiveness of Ara C to induce liver toxicity was observed as the liver is the primary target organ for pharmaceuticals, xenobiotics, and other harmful substances (Gupta *et al.,* 2022).

Elevation levels of liver enzymes such as ALT, AST, and ALP are signs of hepatic injury and dysfunction (Rjeibi *et al.,* 2016; Thakur *et al.,* 2024). The current study revealed that the administration of Ara C to rats significantly increased the levels of ALT, AST, and ALP. These results accord with the conclusions stated by Majed, (2022) and Faruqi & Tadi (2023), this might be attributed to the accumulation of Ara C in liver tissues leads to cell membrane disintegration, resulting in the release of enzymes into the bloodstream. Interestingly, the TTE treatment resulted in a reduction of liver enzymes AST, ALT, and ALP.

The results suggest that the TTE and its polyphenolic bioactive components may mitigate liver damage caused by Ara C by preserving the integrity of liver cell membranes. Multiple studies have demonstrated that utilizing different plant extracts as a reservoir of natural antioxidants can diminish the adverse effects and toxicity of Ara C (Ara *et al.,* 2022; Kolure *et al.,* 2023; Foghis *etal.,* 2023; Datta *et al.,* 2023; Thilagavathi *et al.,* 2023; Chen & Ding, 2024).

In a study conducted by Akbary *et al.* (2022), it was found that the consumption of a methanolic extract of TTE at doses of 0.5, 1, and 1.5 mg/kg diet resulted in improved hepatic and digestive enzymes, as well as lipid profile. In their study (Akbary *et al.,* 2020; and Boota *et al.,* 2022) found that the polyherbal preparation had a hepatoprotective effect by restoring the levels of ALT, AST, ALP, and bilirubin,

which are crucial indicators in assessing liver function. The resistance or toxicity of Ara C seems to be linked to the intracellular buildup and persistence of Ara-CTP, which is caused by genetic differences related to metabolic enzymes (Di Francia *et al.,* 2021).

Furthermore, Almasi *et al.,* (2017) conducted that supports our findings, they revealed that administering TT hydroalcoholic extract to rats with non-alcoholic fatty liver at dosages of 500, 750, and 1000 mg/kg resulted in decreased levels of ALT, ALP, and AST, as well as improved necrosis and liver fat levels. Moreover, the presence of saponins in TTE inhibits the process of arachidonic acid metabolism, leading to a decrease in levels of reactive oxygen species and lipid peroxides (Guo *et al.,* 2007).

The flavonoids found in TTE have been shown to have hepatoprotective qualities (Kiani *et al.,* 2022), as evidenced by several studies (Seevola *et al.,* 1984; Wegener and Fintelmann, 1999; Rahmathulla *et al.,* 2012).

4.2.2. Assessment the Activity of Oxidant and Antioxidant Enzymes

The results of serum conceutration of MDA,CAT and SOD of male rat were give TTE and Ara –c for 4 weeks show in ***Chart*** (4.2) .

Firstly, the Ara C treated group's serum MDA level was significantly higher than the control group's (P< 0.0001). ***Chart (4.2***)shows that the MDA level of animals treated with Ara C was significantly higher (2.78±0.2) than that of the control group (1.16±0.2) based on post-hoc analysis using Tukey's multiple comparisons test. Furthermore, there was no discernible difference (P=0.94) between the TTE-treated male rats and the control group.

It's interesting to note that after receiving TTE+ Ara C (2.09±0.2) compared to the Ara C treated group (2.78±0.2), the MDA level was significantly lower (P<0.0001) ***Chart* (*4.2*).**

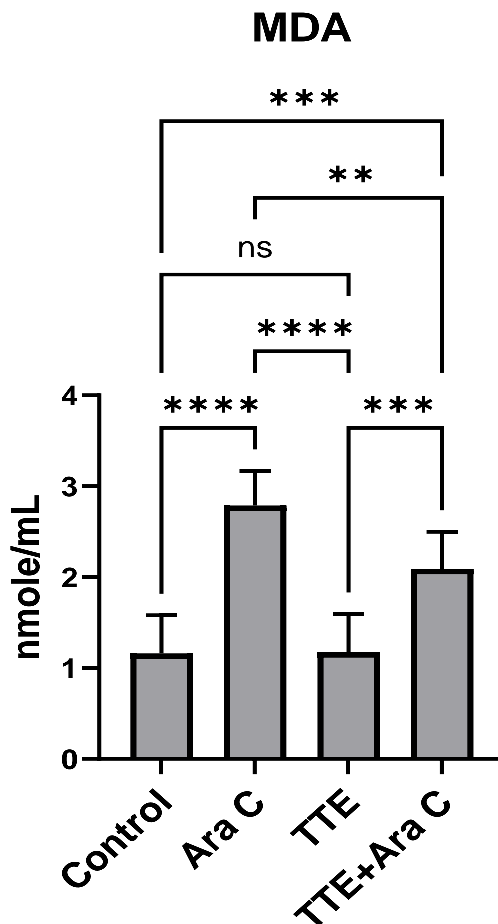


Chart **4.2: Effect of TTE and Ara C on the serum of oxidant enzyme in male rats**

Effect of TTE and Ara C on serum oxidant enzymes in male rats for 4 weeks. Treated animals with 25 mg/kg. BW IP of Ara C showed a significant increase (****P < 0.0001) in MDA compared to control negative group (treated with normal saline). Male rats treated orally with 250mg/kg. BW of TTE have shown no significant effect in MDA compared to control negative group. A combination of TEE+Ara C significantly decreased (****P < 0.0001) in MDA comparison with control positive group. *: values significantly different (P= 0.01). **: values significantly different (P= 0.003). Data are expressed as mean ± SEM, n = 5.

Second, when TTE and Ara C were given to male rats, studies were carried out to find out the amount of serum antioxidant enzymes CAT and SOD. When comparing the Ara C treated group to the control group, the level of both antioxidant enzymes in the serum was significantly lower ($P<0.0001$).

Using Tukey's multiple comparisons test for post-hoc analysis, the results showed that animals treated with Ara C had significantly lower levels of SOD (79.96±12.97) and CAT (0.79±0.04) compared to the control group's levels of SOD (191.8±12.97) and CAT (0.99±0.04) ***Chart*** (***4.3***). Furthermore, there was no discernible difference ($P=0.99$) between the TTE-treated male rats and the control group.

Interestingly, the level of both antioxidant enzymes was significantly increased ($P<0.0001$) following administration of the TTE+ Ara C (CAT: 0.98±0.04; SOD: 184.2 ±12.97) in comparison with Ara C treated group (CAT: 0.79±0.04; SOD: 79.96±12.97) ***Chart*** (***4.3***).

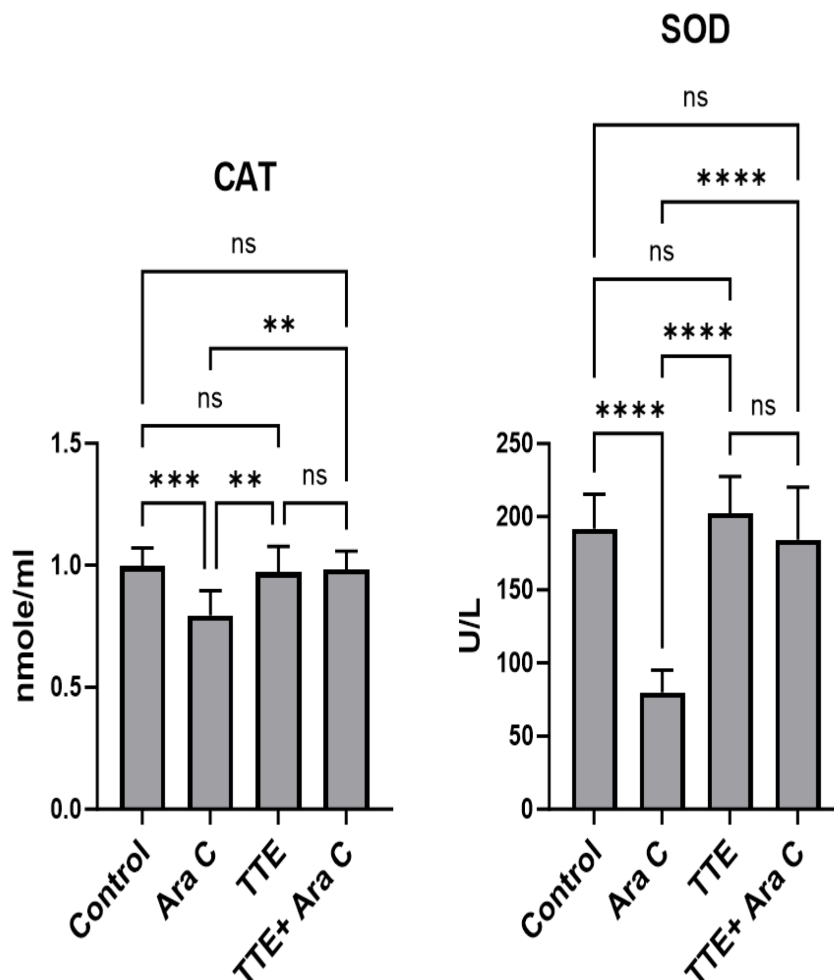


***Chart* 4.3: Effect of TTE and Ara C on the serum of antioxidant enzymes in male rats**

Effect of TTE and Ara C on antioxidant enzymes in male rats for 4 weeks. Treated animals with 25 mg/kg. BW IP of Ara C showed a significant decrease (****P < 0.0001) in CAT and SOD compared to control negative group (treated with normal saline). Male rats treated orally with 250mg/kg. BW of TTE have shown no significant effect in CAT(p=0.99) and SOD(p=0.84) compared to control negative group. A combination of TEE+Ara C significantly increased (****P < 0.0001) in CAT and SOD comparison with control positive group. *: values significantly different (P= 0.01). **: values significantly different (P= 0.003). Data are expressed as mean ± SEM, n = 5.

The present findings indicate that the administration of Ara C resulted in significant decrease oxidative stress, as evidenced by elevated levels of oxidative parameters MDA and reduced levels of antioxidant defense parameters CAT and SOD. Ara C induces hepatic lipid peroxidation in rats and reduces cellular stocks of antioxidants, including both enzymatic and non-enzymatic antioxidants. This leads to oxidative stress and tissue damage and dysfunction caused by free radicals. Chemotherapeutic immunosuppressive, cytotoxic drugs with reported adverse effects, including oxidative damage to testis (Abdul-Hamid *et al.,* 2023; Alharbi, 2024; Biyik *et al.,* 2024).

According to Mottola *et al.* (2024), oxidative stress biomarkers in male infertility. The results of our study were consistent with prior research, which demonstrated an increase in blood MDA and NO levels, as well as a decrease in antioxidant enzymes, following exposure to Ara C (Majed, 2022).

In contrast, when compared to the Ara C group, the oral administration of the TTE with Ara C led to a significant increase in liver CAT and SOD levels, as well as a notable decrease in liver MDA levels. These results align with the findings reported by Amin *et al.* (2006) which demonstrated a significant increase in liver CAT and SOD levels and a decrease in MDA levels in diabetic rats treated with TTE. This may be attributed to the high concentration of flavonoid molecules with possible antioxidant activity, which is consistent with the findings of (Fatima *et al.,* 2015; and Ali *et al.,* 2018).

A study conducted by Al-Eisa *et al.* (2022) showed that administering a dose of 10 mg/kg of hydroalcoholic extract of TTE to type 2 diabetic rats resulted in higher levels of superoxide dismutase and glutathione peroxidase, decreased levels of malondialdehyde, and reduced necrosis and apoptosis in the liver tissue.

Maintaining an equilibrium between antioxidants and free radicals is crucial for effectively removing oxidative stress in intracellular organelles because antioxidants are the primary defense against free radicals, limiting their toxicity (De Luca *et al.,* 2021; Hong *et al.,* 2024; Mottola *et al.,* 2024).

In pathological circumstances such as testes, the production of ROS can disrupt the balance and put an additional burden on the antioxidant system (Sengupta *et al.,* 2024; and Sudhakaran *et al.,* 2024).

Free radical scavenging enzymes including SOD, CAT, GPx, GRx, and GST are the first line of defense against oxidative stress (Sadiq, 2023; Krishnamurthy *et al.,* 2024; Alam *et al.,* 2024).

Increased lipid peroxidation leads to decreased enzyme levels CAT and SOD (Palla & Ahmed, 2024). In testes, superoxide radicals at the site of damage reduce the activity of SOD and catalase, causing superoxide anion buildup and testes damage.

Ara C therapy is related with a decrease in the activity of the antioxidant system, resulting in increased oxidative stress, which is the most prevalent cause of sperm destruction. Ara C-induced cell death was reduced by treatment with multiple distinct free-radical scavengers non enzymatic (N-acetyl-l-cysteine, dipyridamole, uric acid, and vitamin E) and was increased following depletion of cellular glutathione levels (Geller *et al.,* 2001).

Reactive oxgen species such as (superoxide anion radicals) are neutralized by cellular antioxidant defenses which include superoxide dismutase (SOD) and glutathione peroxidase (GPX) (Jomova *et al.,* 2024).

Superoxide dismutase (SOD) is an enzyme that transforms superoxide radicals to hydrogen peroxide and O2, thereby halting the negative effects of a radical chain reaction at the start stage (Han *et al.,* 2024; and Uniyal *et al.,* 2024).

Moreover, Ali *et al.* (2018) reported that co-administration of TTE significantly alleviated MDA and NO values in the treated group compared to Alpha-Cypermethrin-induced hepatotoxicity in rats group.

4.3. Hormonal Parameters

4.3.1. Measuring of the Sex Hormone and 17β-HSD Levels in Male Rats

Experiments were conducted to assess the level of serum sex hormones and 17β-HSD levels following administration of TTE and Ara C in male rats. The level of serum sex hormones. testosterone, Ara C were significantly decreased in all examined sex hormones i.e. testosterone level (1.88±0.36), FSH level (1.66±0.36), LH level (3.96±0.57), and 17β-HSD (11.21± 0.71) as compared to control group testosterone level (5.23±0.36), FSH level (4.08±0.36), LH level (8.06±0.57), and 17β-HSD (16.06±0.71) ***Chart*** (***4.4***). In addition, male rats treated with TTE have shown no significant effect (testosterone: P= 0.78; FSH: P=0.85; LH: P=0.72; 17β-HSD: P= 0.51) as compared to control group.

Interestingly, testosterone, FSH, LH, and 17β-HSD levels were significantly increased ($P<0.0001$) following administration of the TTE + Ara C (testosterone: 4.09±0.36; FSH: 3.29±0.36; LH: 6.72±0.57; 17β-HSD: 14.16±0.71) in comparison with Ara C treated group (testosterone: 1.88±0.36; FSH: 1.66±0.36; LH: 3.96±0.57; 17β-HSD: 11.21±0.71) ***Chart*** (***4.4***).

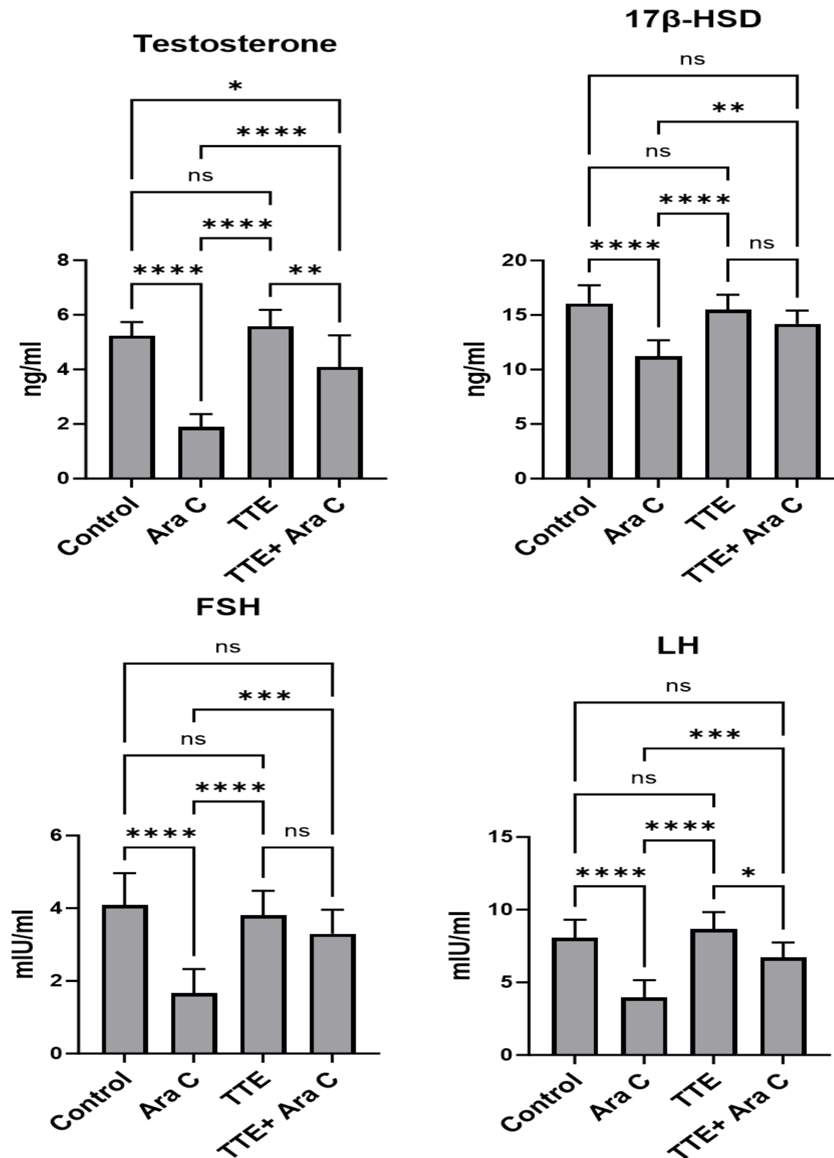


Chart 4.4: Effect of TEE and Ara C on the level of sex hormones and 17β-HSD in male rats

Effect of TTE and Ara C on serum testosterone,FSH,LH and 17β-HSD in male rats for 4 weeks. Treated animals with 25 mg/kg. BW IP of Ara C showed a significant decrease (****P < 0.0001) in testosterone,FSH,LH and 17β-HSD compared to control negative group (treated with normal saline). Male rats treated orally with 250mg/kg. BW of TTE have shown no significant effect in all sex hermons and 17β-HSD. compared to control negative group. A combination of TEE+Ara C significantly increased (****P < 0.0001) in testosterone,FSH,LH and 17β-HSD comparison with control positive group. *: values significantly different (P= 0.01). **: values significantly different (P= 0.003). Data are expressed as mean ± SEM, n = 5.

Male reproductive toxicity was induced in rats with the oral administration of cytarabine. The toxic effect of it can be identified by decreased levels of serum testosterone, follicle-stimulating hormone and luteinizing hormone as well as reduced levels of 17β-Hydroxysteroid dehydrogenase. Additionally, it leads to decreased semen quantity and quality, and increased caspase levels compared to other groups. The effects mentioned were consistent with those documented in prior reports.

The findings of the present study clearly showed that Ara C therapy inhibits the process of spermatogenesis and has a negative impact on the growth and functioning of the testes in rats. This is achieved by reducing the proliferation of germ cells and inducing apoptosis. The regulation of spermatogenesis is influenced by the microenvironment around germ cells. Under typical circumstances and in reaction to inflammatory triggers, somatic cells (specifically Sertoli cells, Leydig cells, and peritubular cells) release proinflammatory and immunoregulatory cytokines, along with growth factors, that control the proliferation, differentiation, and programmed cell death of SSCs (Weinbauer *et al.,* 2010; Potter and DeFalco, 2017).

Tribulus terrestris extract was identified to mitigate all the reproductive disturbances caused by Ara C. The findings of our study correspond with those of (Qureshi *et al.,* 2014), demonstrating that TTE can effectively boost testosterone levels.

We observed a notable rise in serum testosterone levels following TTE administration, this results agreement with Roaiah *et al.* (2017). The release of nitric oxide by TTE may provide a reasonable explanation for the observed physiological responses to TTE supplementation, regardless of the testosterone level. A study conducted by (Miranda *et al.*, (2022) discovered that both TTE and L. meyenii have beneficial effects on blood testosterone levels, sperm concentration, and epididymal shape. *Tribulus* has been recognized as a potential stimulator of testosterone synthesis

(Gauthaman *et al.,* 2006), primarily attributed to the steroidal saponins found in this plant. These compounds have the ability to influence sexual performance and address various sexual disorders by modulating sexual energy and strength through an elevation in free testosterone levels in males (Pavin *et al.,* 2018 and Shawish *et al.,* 2021).

According to reports Karimi *et al.* (2012) and Pavin *et al.* (2018), TTE has been found to cause toxicity in the reproductive system of male rats, as observed through immunohistochemical and pathophysiological effects. This can be attributed not only to its antioxidant action but also to its ability to stimulate the production of testosterone from the leydig cell. An analysis of the phytochemicals found in the medicinal plant *Tribulus terrestris* has revealed the presence of various bioactive compounds, including saponins, alkaloids, flavonoids, and sterols. These compounds have been shown to possess antioxidant (Hamzah and AL-Musawi, 2023), anti-tumor (Abdel-All *et al.,* 2021), and anti-apoptotic properties for cells (Keshtmand *et al.,* 2014; Almasi *et al.,* 2017; Naseri *et al.,* 2019).

Tribulus terrestris extract (TTE) has been shown to enhance sperm production and increase testosterone levels in males (Sirotkin and Kolesarova, 2021; Da Silva *et al.,* 2023). It has been shown that increasing the concentration of testosterone hormones in females can have a minor effect on boosting reproductive function (Liu *et al.,* 2021).

4.4. Epididymal Sperm Parameters

Experiments were conducted to assess the activity of epididymal sperm following administration of TTE and Ara C in male rats. The activity of epididymal sperm parameters i.e. sperm's motility, integrity, VCL, and vigor were significantly decreased in the Ara C treated group compared to control group ($P<0.0001$).

Post-hoc analysis using Tukey's multiple comparisons test indicated that animals treated with Ara C were significantly decreased in all examined sperm parameters i.e. motility (21.31±1.86), integrity (20.61±1.69), VCL (63.42±2.54), and vigor (2.56±0.21) as compared to control group motility (41.14±1.86), integrity (35.83±1.69), VCL (83.71±2.54), and vigor (4.16±0.21) ***Chart (4.5***). In addition, male rats treated with TTE have shown no significant effect (motility: P= 0.99; integrity: P=0.99; VCL: P=0.88; vigor: P= 0.92) as compared to control group.

Interestingly, sperm's motility, integrity, VCL, and vigor were significantly increased ($P<0.0001$) following administration of the TTE + Ara C (motility: 37.29±1.86; integrity: 34.72±1.69; VCL: 81.45±2.54; vigor: 4.27±0.21) in comparison with Ara C treated group (motility: 21.31±1.86; integrity: 20.61±1.69; VCL: 63.42 ± 2.54; vigor: 2.56±0.21) ***Chart (4.5***).

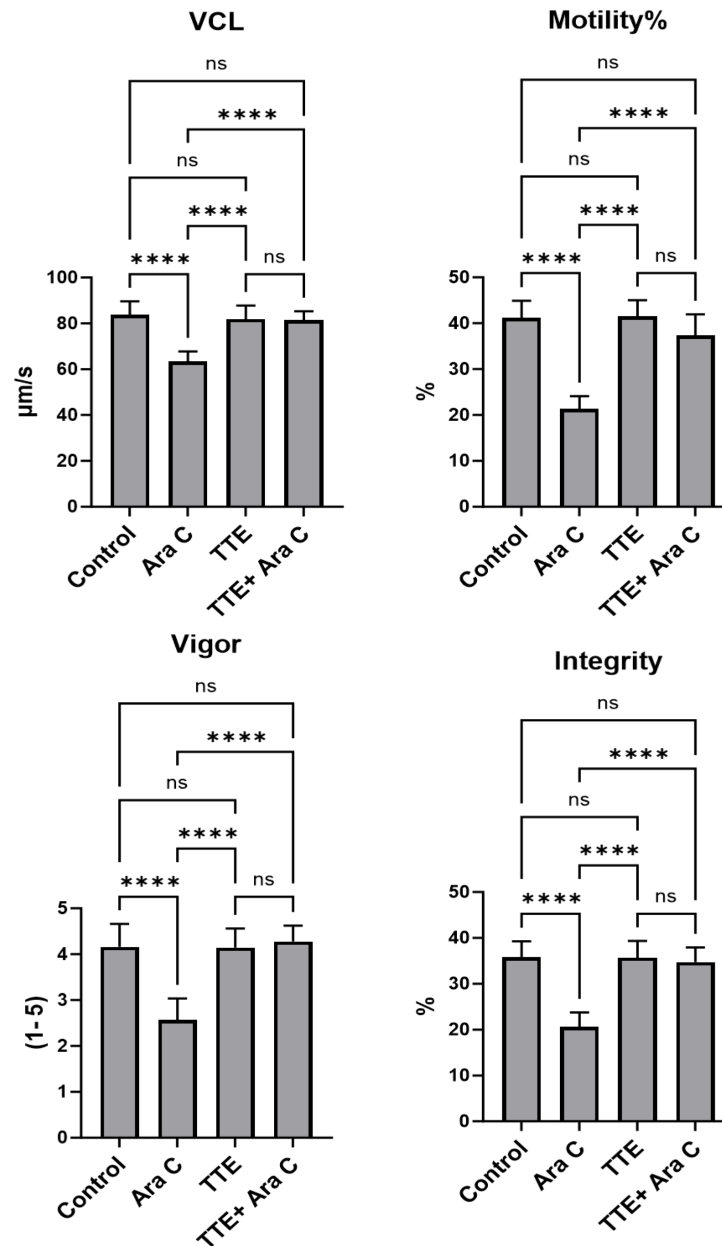


Chart 4.5: Effect of TEE and Ara C on the activity of sperm in male rats

Effect of TTE and Ara C on serum sperm s motility, integrity, velocity of the curved line and vigor in male rats for 4 weeks. Treated animals with 25 mg/kg. BW IP of Ara C showed a significant decrease (****P < 0.0001) in sperm s motility, integrity, velocity of the curved line and vigor compared to control negative group (treated with normal saline). Male rats treated orally with 250mg/kg. BW of TTE have shown no significant effect in all sperm s motility, integrity, velocity of the curved line and vigor . compared to control negative group. A combination of TEE+Ara C significantly increased (****P < 0.0001) in sperm s motility, integrity, velocity of the curved line and vigor comparison with control positive group. *: values significantly different (P= 0.01). **: values significantly different (P= 0.003). Data are expressed as mean ± SEM, n = 5.

To examine the effect of Ara C on the generation of sperm, we sacrificed the rats from all treated groups at the last possible time point of their survival (28 days' post treatment) and isolated sperm from their epididymis. Our results show decrease of sperm parameters from Ara C -treated groups compared to the control group. This could be attributed to the capacity of Ara C and other chemotherapies to target rapidly proliferating cells.

These findings are consistent with prior research that found Ara C impairs spermatogenesis, increases the percentage of tubules containing apoptotic cells, and lowers sperm parameters in adult mice (Namoju *et al.,* 2014; and Michailov *et al.,* 2021).

Excessive oxidative stress can affect the reproductive system as well as features of the sperm (Muñoz *et al.,* 2024), such as sperm concentration, motility, and morphology, resulting in a decline in semen quality and a low conception rate (Shiva *et al.,* 2011; Alahmar, 2019; Fang & Zhong, 2020; Aitken & Drevet, 2020).

The sperm plasma membrane contains polyunsaturated fatty acids, making it more sensitive and susceptible to oxidative damage, and spermatozoa eventually lose their ability to fertilize (Lenzi *et al.,* 1996; Aitken *et al.,* 2022; Bhattacharya *et al.,* 2024).

Adult leukemia patients had a significantly lower sperm count, concentration, and motility than healthy donors and other adult cancer patients (Hallak *et al.,* 1999; Johnson *et al.,* 2013; Ku *et al.,* 2015).

Chemotherapies typically cause gonadotoxicity because they target actively proliferating cells (Howland, 2009; Delessard *et al.,* 2020; Essawy *et al.,* 2024). Chemotherapy's negative effect on spermatogenesis is determined by a number of

variables that involve the type of treatment, dosage, beginning semen quality, and therapy regimen (Giwercman and Petersen, 2000).

A prior investigation on rats found that injecting Ara C (intraperitoneal; i.p) into four-week-old rats disrupted spermatogenesis and had a detrimental impact on testicular growth and function by generating DNA damage and apoptosis and decreasing germ cell proliferation, furthermore, the treatment group had a lower sperm count than the control group (Namoju *et al.,* 2014).

One of the first and most significant effects of oxidative stress to be identified was loss of motility (Ribeiro *et al.,* 2022). Antioxidants like melatonin (Bameri *et al.,* 2024; and Qi *et al.,* 2024), genistein, carnitine, or α-tocopherol can help mammalian spermatozoa sustain function after cryostorage, indicating a link between lipid peroxidation and motility. (Thomson *et al.,* 2009; Ashrafi *et al.,* 2013; Gibb *et al.,* 2015; Longobardi *et al.,* 2017).

4.5. Assessment the Activity of Caspase 3 (Apoptotic Test)

The results of serum conceutration of caspase 3 of male rats were give TTE and Ara-c for 4 weeks show in ***Chart*** (4.6) .

Post-hoc analysis using Tukey's multiple comparisons test indicated that animals treated with Ara C were significantly increased in the activity of caspase enzyme (2.09±0.17) as compared to control group (0.61±0.17) ***Chart (4.6***). In addition, male rats treated with TTE have shown no significant effect (P=0.21) as compared to control group.

Interestingly, caspase level was significantly reduced (P<0.0001) following administration of the TTE+ Ara C (0.99±0.17) in comparison with Ara C treated group (2.09±0.17) ***Chart (4.6***).

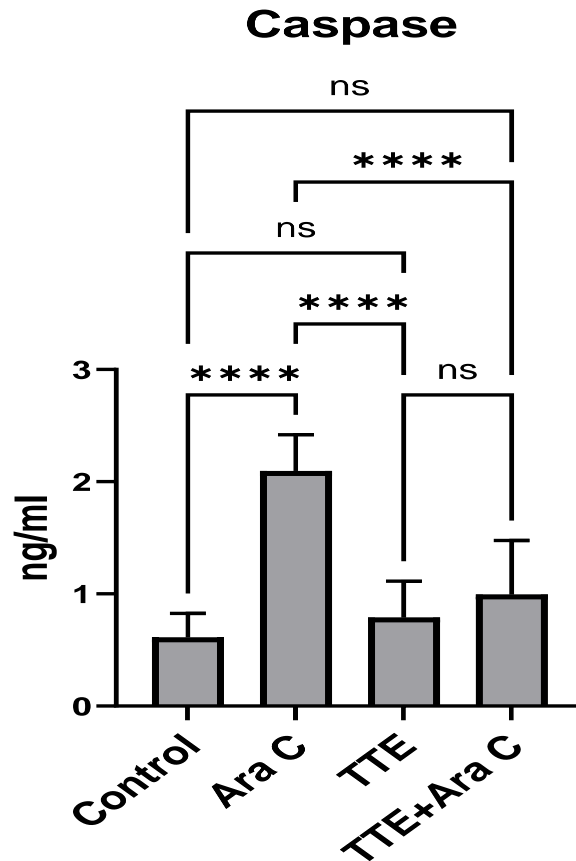


Chart (4.6): Effect of TTE and Ara C on the serum of caspase 3 enzyme in male rats

Effect of TTE and Ara C on serum caspase 3 enzyme in male rats for 4 weeks. Treated animals with 25 mg/kg. BW IP of Ara C showed a significant increase (****P < 0.0001) in the level of caspase 3 enzyme compared to control negative group (treated with normal saline). Male rats treated orally with 250mg/kg. BW of TTE have shown no significant effect in caspase 3 enzyme. compared to control negative group. A combination of TEE+Ara C significantly dcreased (****P < 0.0001) in caspase 3 enzyme comparison with control positive group. *: values significantly different (P= 0.01). **: values significantly different (P= 0.003). Data are expressed as mean ± SEM, n = 5.

Several investigations studied the effects of Ara C on human reproduction, and detailed reviews addressed the strength of the evidence on Ara C toxicity.

The current study's findings clearly demonstrated that Ara C therapy impairs spermatogenesis and adversely influences testicular development and function in rats by lowering germ cell proliferation and producing apoptosis. The microenvironment around germ cells is important in regulating spermatogenesis (Aitken and Lewis, 2023).

Under normal conditions and in response to inflammatory stimuli, somatic cells (Sertoli cells, Leydig cells, and peritubular cells) secrete proinflammatory and immunoregulatory cytokines, as well as growth factors, which regulate SSC proliferation/differentiation and apoptosis (Weinbauer *et al.,* 2010; and Potter and DeFalco, 2017).

This may indicate Ara C important effect on spermatogenesis, as well as the mechanism of Ara C being an essential factor. However, this mechanism differs in some function of spermatogenesis (cellular apoptosis).

Our results are in agreement with previous reports Keshtmand *et al.* (2014), that demonstrated of TT extract has been shown to protect against cisplatin-induced apoptosis of the testis and seminiferous tubules. This may be due to the presence of antioxidants that operate through several central and peripheral mechanisms, which have been shown to protect against cisplatin-induced apoptosis of the testis and seminiferous tubules. This may be due to the presence of antioxidants that operate through several central and peripheral mechanisms.

High Ara C intake promotes apoptosis of Leydig cells and germ cells, resulting in an undeveloped testis with histopathological alterations such as atrophied seminiferous tubules, fewer late spermatids, and more karyopyknotic cells. Reduced testicular weight and altered spermatogenesis (Yue *et al.,* 2011).

Geller *et al.* (2001) found that Ara C-induced apoptosis in cultured cerebral cortical neurons is caused by oxidative stress.

It has been observed that the apoptotic process can proceed regardless of the activation of inflammation, cytokines, or ROS generation (Hussain *et al.,* 2023).

Spermatozoa apoptosis when an enzyme called phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) is activated, where cells are used for survival (Koppers *et al.,* 2011). When the PI3K signaling pathway is activated, it phosphorylates downstream kinases such as AKT (Protein Kinase B), resulting in gametes that are both active and viable (Koppers *et al.,* 2011). Notably, spermatozoa have various pro-survival hormone receptors, including as prolactin (Iancu *et al.,* 2023) and insulin, which, when triggered by their respective ligands, allow for ongoing survival. In contrast, if the PI3K inhibitor wortmanin is administered, gametes rapidly enhance mitochondrial ROS production, making cells more sensitive to apoptosis (Koppers *et al.,* 2011).

The oxidants damage the spermatozoon membrane (Zahid *et al.,* 2024), resulting in an oxidative burst in which the oxidant/antioxidant ratio is dramatically altered (El-Tohamy, 2012; Bisht & Dada, 2017; Hussain *et al.,* 2024; Toprak & Kulaksiz, 2024).

Oxidation readily targets spermatozoa, spermatogenic cells use apoptosis to remove oxidative DNA via p53-dependent and -independent processes (Jana *et al.,* 2010; Eleawa *et al.,* 2014; Sharma *et al.,* 2023), indicating that increased activity may contribute to male infertility (Khokhlova *et al.,* 2020; and Men *et al.,* 2023).

Furthermore, ROS activates caspases and nucleases that stimulate apoptotic pathways, resulting in indirect damage to sperm DNA via abortive apoptosis (Bejarano *et al.,* 2012; Bui *et al.,* 2018; Aitken & Lewis, 2023).

4.6. Histopathological Changes

4.6.1. Histopathological Examination of the Testis and Epididymis

Histological analysis revealed that Ara C caused damage in the testes, as evidenced by an increase in cell death and a decrease in seminiferous tubule growth. In comparison to the control group ***Chart*** (1), the histopathological images showed that the seminiferous tubules of the Ara C treated group had considerable cellularity together with degenerative alterations and parenchymal congestion with focal degeneration ***Chart*** (2).

While the histolopathogical images of the group treated with TTE and Ara C show no substantial degradation and a relative increase in cellularity with a few mature sperm positioned in the center image (3). There is a noticeable increase in spermatogenic activity in this group as compared to the fourth, and there is more mature sperm in this group ***Chart*** (4).

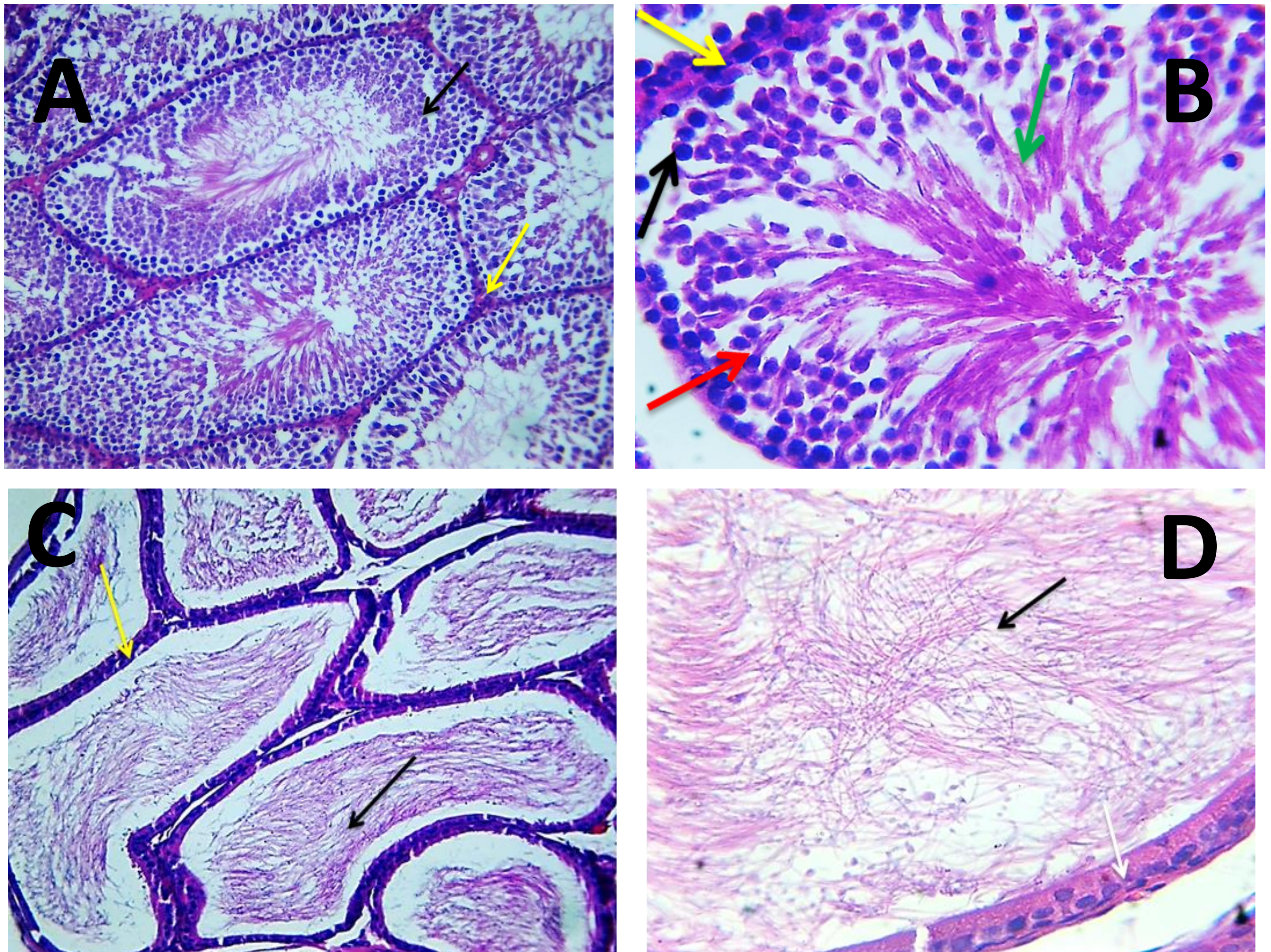


Chart (4.7) Histological examination of a control rat testicular tissue. (**A**) showing the normal architecture of testicular tissue, significant rounded to oval shaping of seminiferous tubules (black arrow) , marked and normal surrounding Leydig cells (yellow arrow).(H and E, 10X). **(B)** Histological examination of a control rat testes showing the normal structure of seminiferous tubules, normal germinal epithelium , regular spermatogonia (black arrow) , typical surrounding Leydig cells (yellow arrow) ,remarkable spermatocytes (red arrow) and significant long spermatid (green arrow) .(H and E, 40X). **(C)** Histological examination of a control rat epididymis showing the normal histoarchitectural appearance of epididymal tubules (yellow arrow), normal spermatic density (black arrow) .(H and E, 10X). **(D)** Histological examination of a control rat epididymis showing the normal histological structure of epididymal tubule, significant high spermatic density (black arrow)with normal lining endothelia(white arrow) .(H and E, 40X).

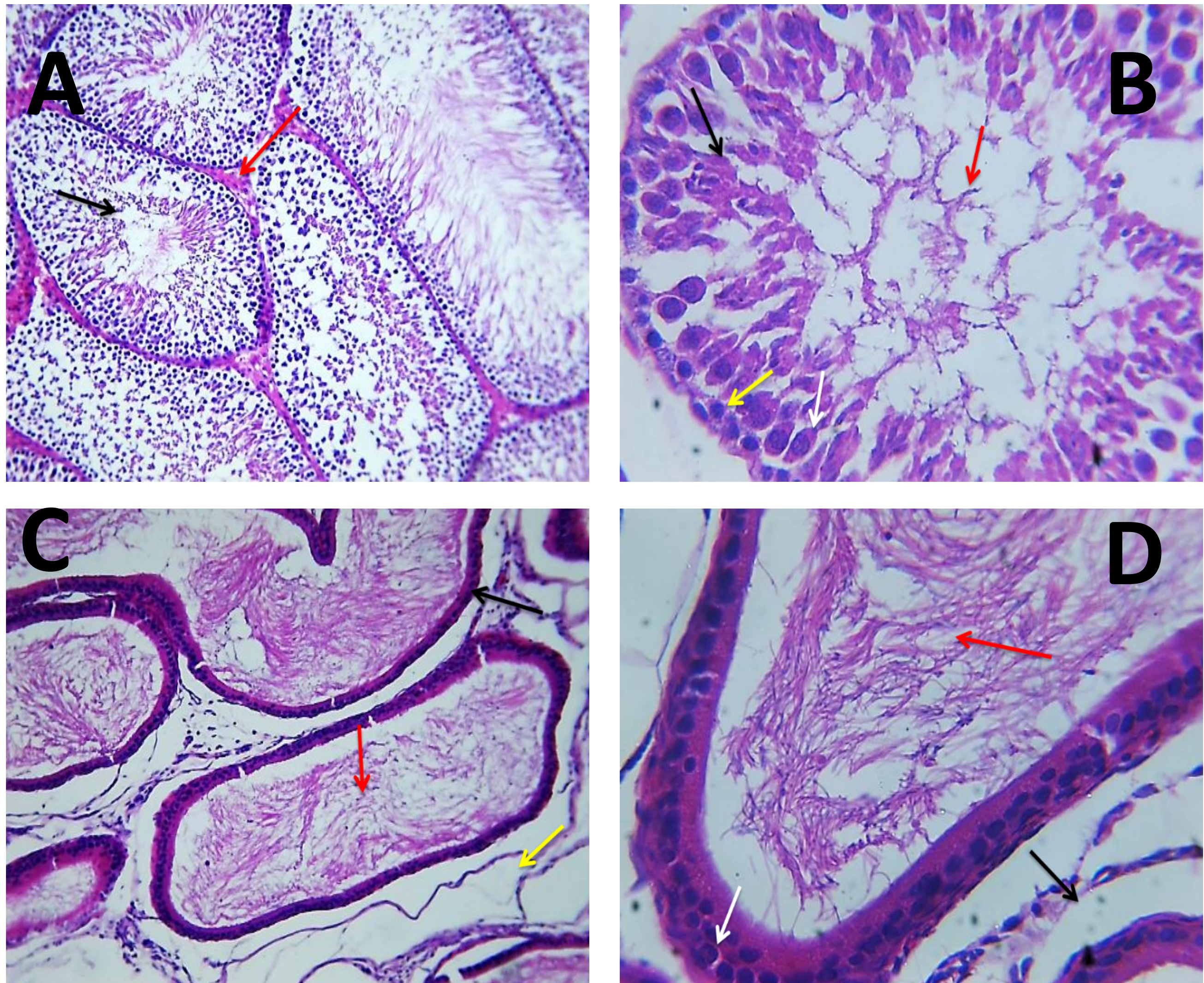


Chart (4.8) Histological examination of testicular tissue for TTE treated animal revealing the **(A)** normal structure of testicular tissue (black arrow), with blood vessels congestion (red arrow).(H and E, 10X) **(B)** showing the normal structure of a seminiferous tubule (black arrow), with mild germinal epithelia vacuolation (red arrow), noticeable newly formed spermatocytes (white arrow) and normal spermatogonia (yellow arrow), (H and E, 40X). **(C)** showing the normal epididymal tubules morphology(black arrow), regular spermatic density (red arrow) , with widening in interstitial spaces (yellow arrow).(H and E, 10X).**(D)** Histological examination of TTE treated rat epididymal tubule revealing normal histology, high spermatic density (red arrow) , with widening in interstitial spaces (black arrow) and normal epithelial lining (white arrow) .(H and E, 40X).

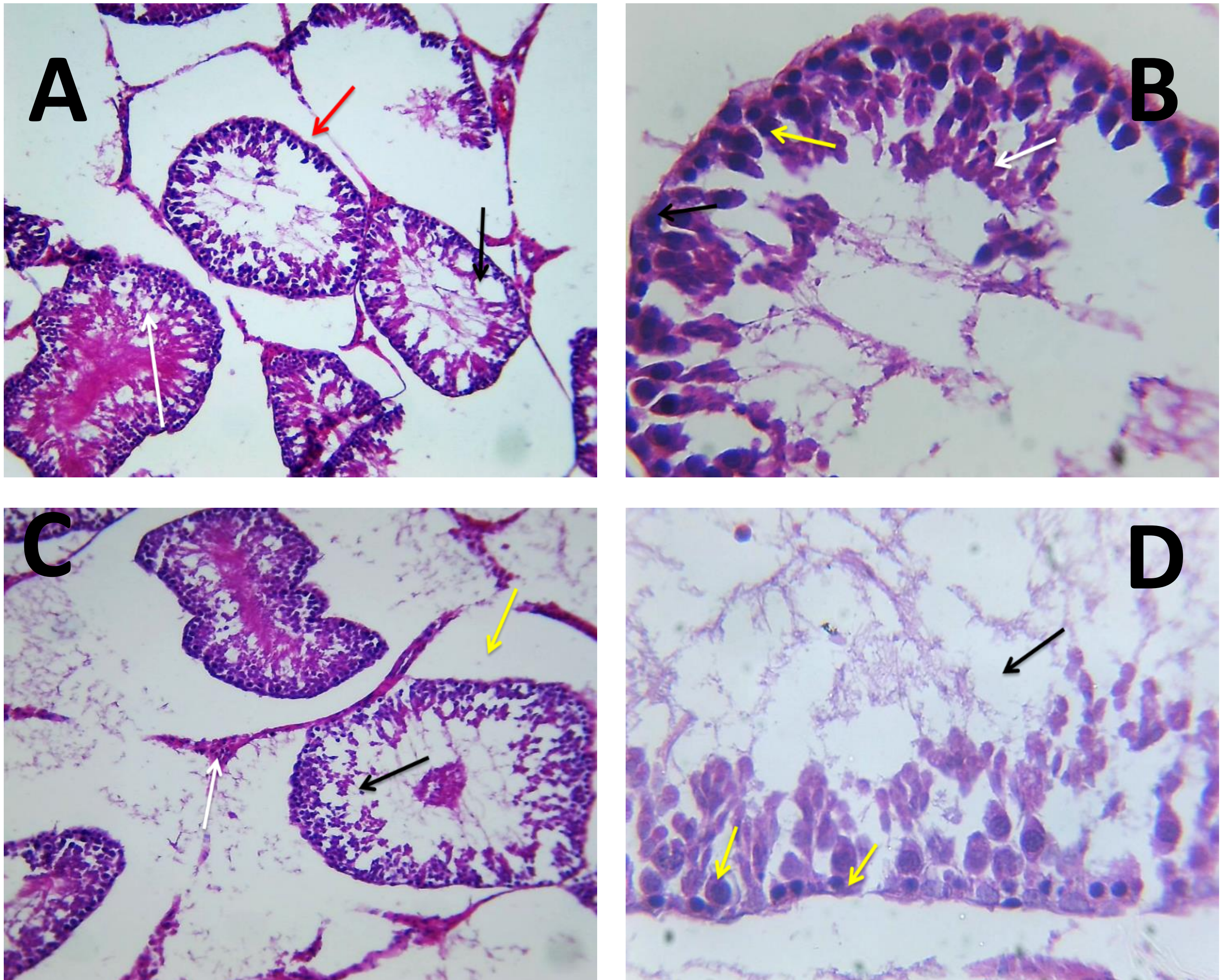


Chart (4,9) Histopathological examination of testicular tissue for Ara-C treated rat(**A**)_showing characteristic histopathological alterations manifested by, marked seminiferous tubules atrophy (red arrow) , with severe widening in interstitial spaces (black arrow) and vacuolated epithelial lining (white arrow) .(H and E, 10X). **(B** showing characteristic histopathological alterations in seminiferous tubule, few spermatogonia (black arrow) with pyknotic spermatocytes(yellow arrow) and vacuolated primary spermatids (white arrow) .(H and E, 40X). **(C)** showing significant histopathological alterations in germinal epithelium, sever spermatid degeneration (black arrow) with atrophied tubules (yellow arrow) and congested interstitial spaces with dilation (white arrow) .(H and E, 10X). **(D)** showing significant histopathological alterations in germinal epithelium, sever spermatid degeneration and vacuolation (black arrow) , few spermatogonia and spermatocytes (yellow arrow) .(H and E, 40X).

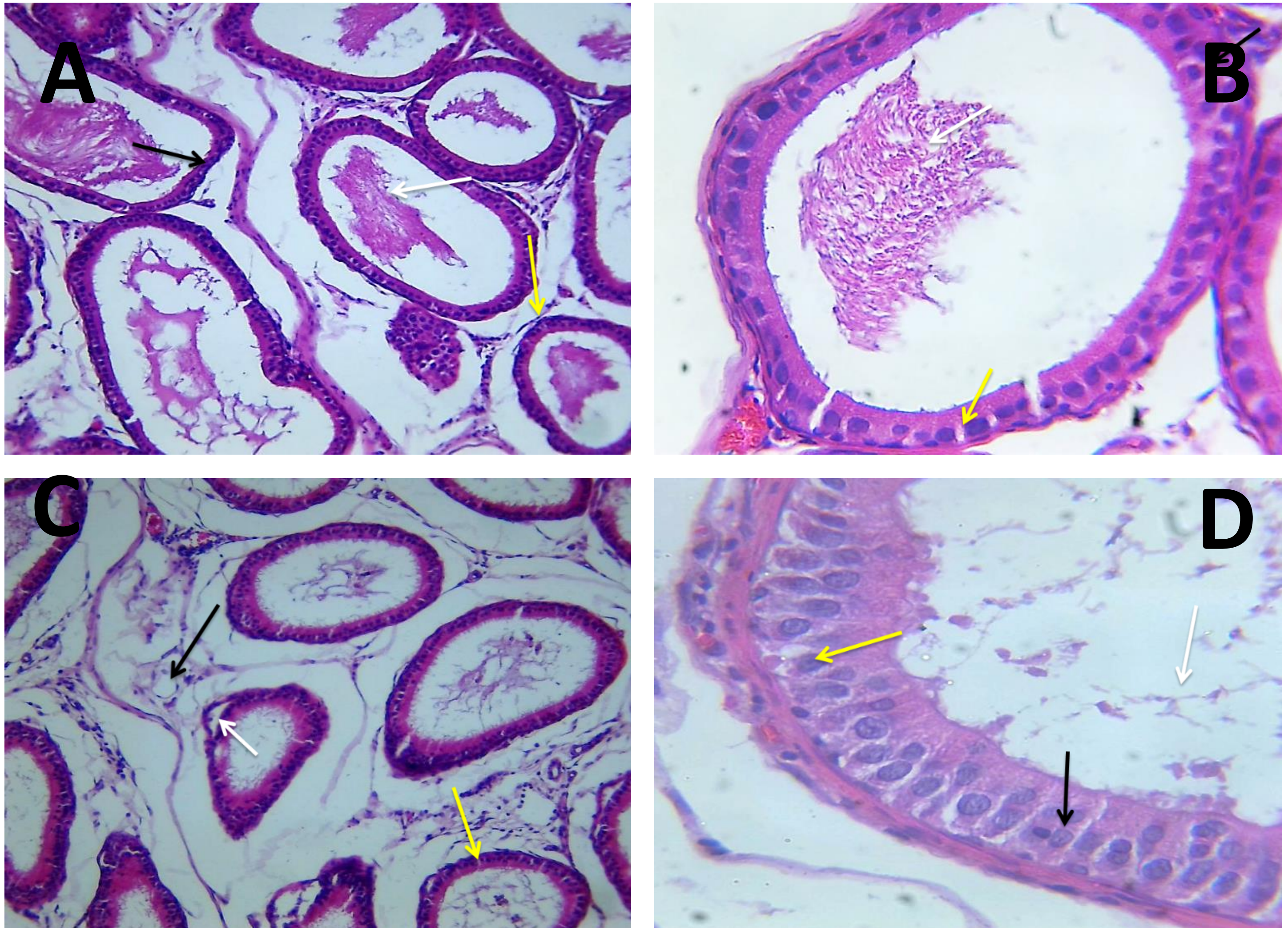


Chart (4.10) Histological examination of epididymis for Ara-C treated rat (**A**) showing significant histopathological changes , marked increase in interstitial spaces (black arrow) with atrophied tubules (yellow arrow) and low sperm density in the lumen of the tubule (white arrow) .(H and E, 10X). **(B)** showing significant histopathological changes increase in epithelial lining thickening (black arrow) with few vacuolation (yellow arrow) and low sperm density in the lumen of the tubule (white arrow) .(H and E, 40X). **(C**) showing significant widening in interstitial spaces (black arrow) with atrophied tubules (yellow arrow) and noticeable sloughing in epithelial linning from the basement membrane (white arrow) .(H and E, 10X). **(D**) showing significant increase thickness in epithelial lining (hyperplasia) (black arrow) with few vacuolation (yellow arrow) and low or no sperm density in the lumen of the tubule (white arrow) .(H and E, 40X)

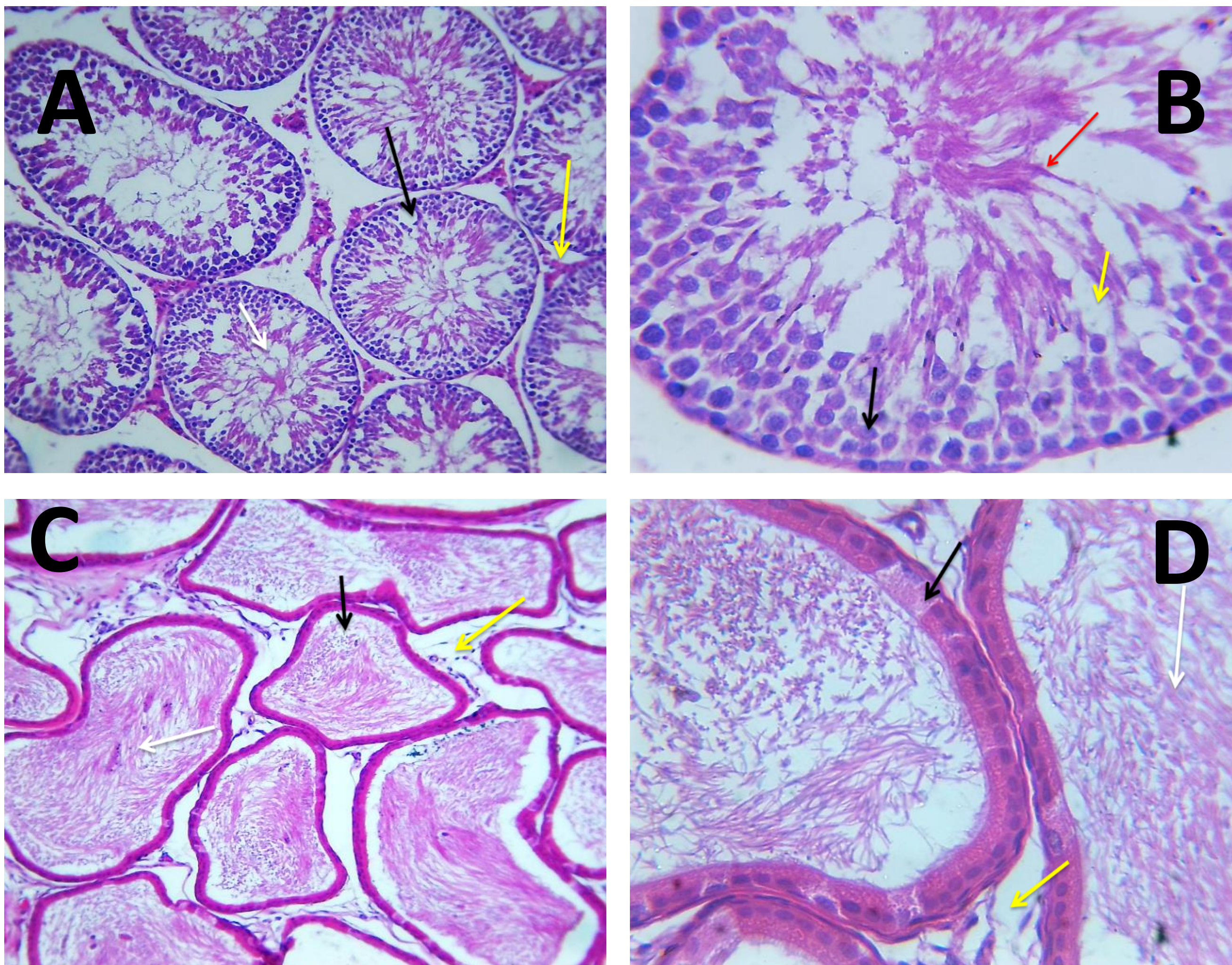


Chart (4.11) Histological examination of testicular tissue for TTE and Ara-C treated rat(**A**) showing significant histological improvements in seminiferous tubules, normal size and rounded morphology (black arrow) with decreased interstitial spaces (yellow arrow) and noticeable normal spermatogenesis(white arrow) .(H and E, 10X). **(B)** showing remarkable regular arranged spermatogonia (black arrow) , few vacuolation in spermatocytes (yellow arrow) with normal spermatid appeared in the lumen (red arrow).(H and E, 40X). **(C)** showing significant histological reversible changes in epididymal tubule (black arrow) with decreased interstitial spaces (yellow arrow) and noticeable normal spermatic density(white arrow) .(H and E, 10X). **(D)** showing significant histological reversible changes in epididymal tubule , normal and regular epithelial lining (black arrow) with decreased interstitial spaces (yellow arrow) and noticeable normal and high spermatic density(white arrow) .(H and E, 40X).

Depending on our findings of the testicular and epididymal histological examination, Ara C therapy greatly reduced proliferative cells two weeks after treatment. Ara C caused damage to the testes, as demonstrated by histological analysis figure (4.a); this was further supported by an increase in cell death and a decrease in cell proliferation in the seminiferous tubules (Niran *et al.,* 2017).

This was explained by the fact that Ara-C and other chemotherapies might target cells that were rapidly growing. (Abadjieva *et al.,* 2019). Additionally, in adult mice, Ara C reduces sperm parameters, increases the proportion of tubules containing apoptotic cells, and impairs spermatogenesis (Howell *et al.,*2001).

In rat testes, Ara C 's histological effects result in minor vacuolization and tubular deformation (Niran *et al.,* 2017). Because of the damage seen in the structure and histology of their seminiferous tubules as well as the observed increase in apoptotic cells, we advise treating rats with Ara C with extra care to ensure their future fertility. Histological examination revealed a definite detrimental effect of Ara C on the cellular composition/structure of the seminiferous tubules.

As a result, we investigated Ara C treatment that affected the apoptotic process and cell proliferation in the treated groups' seminiferous tubules. Histopathological study revealed that Ara C had a definite negative effect on the cellular composition/structure of the seminiferous tubules. As a result, we investigated how all treatments affected the apoptotic process and cell proliferation in the treated groups' seminiferous tubules. Our results revealed an important rise in the percentage of tubules with positive apoptotic cells in the Ara C group 28 days after treatment compared to the control group. Furthermore, histologically, Ara C produces tubular deformation and minor vacuolization in rat testes (Niran *et al.,* 2017).

The significant increase in spermatogenic activity in *Tribulus terrestris* exhibited in Figure (4.4) may be attributed to the androgenic effect of the herb. A vital role that androgens play in the development and differentiation of numerous tissues, including the organs of reproduction, androgen also responsible for pubertal development of testes. Adult rats' testes had significantly more spermatogonia, spermatocytes, and spermatids when *Tribulus terrestris* preparation was administered; these results may indicate that protodioscin stimulated DNA synthesis (Chhatre *et al.,*2014).

According to Abadjieva and Grigorova (Abadjieva *et al.,* 2019), TTE can have an impact on the weight of the rabbits' testicles and body weight. The current study's findings demonstrated that exposure to TTE results in the dilatation of the epididymis, or an increase in cell density. This could be the outcome of a faster rate of cell division, which would enhance the epididymis's ability to store and mature sperm (Asadi *et al.,* 2017).

Furthermore, histologically, our finding agreement with (Namoju *et al.* 2014) found that Ara C produces tubular deformation and minor vacuolization in rat testes.

The histological impacts of cytarabine causes tubular distortion and mild vacuolization in rat testes. Testicular injury with Leydig cells harm related to chemotherapy is one of the most general toxicity consequences of treatment in male (Ibrahim *et al.,* 2017).

Chapter Five: Conclusions and Recommendations

5.1. Conclusions

1. The result pointed the hepatoprotectiv effects of TTE against Ara-c.
2. The role also pointed the reprodevtive effect of TTE against Ara-c in testicular dysfuction manifested by in hancement of sex hermons and testicular tissue and sperm quality.
3. Ara-c have harmfule effect to the hepatic and testicular function and tissue.
4. These observations suggest the use of antioxidant therapies to reduce toxicity of Ara C chemotherapeutic regimens.
5. These findings highlight *T. terrestris'* therapeutic potential and propose its usage as a natural hepatoprotective source and health-promoting product.
6. The present study demonstrates that TTE offered significant protection against Ara C induced testis damage through a unique property of enhancement of antioxidants.
7. Histopathological changes revealed that Ara-C caused damage in the testes and epididymis, as evidenced by an increase in cell apoptosis and a decrease in cell proliferation in the seminiferous tubules.
8. Histopathologically the study showed the testis and epididymis amelioration when combination of TTE with Ara C.

5.2. Recommendations

From the results of the present study, it can be recommended the following**:**

1. Conducting the experiment on other laboratory animals such as rabbits, hamsters…etc.
2. Study the effect of TTE with the same dose of Ara C and study it effect on the kidneys, brain and other organs.
3. Studying the benefits effect of TTE in preserving DNA from the change that occurs as a result of giving a dose of Ara C.
4. More research should be done to evaluate the effects of TTE on cancer diseases.

Chapter Six: References

References

Abadjieva, D., Grigorova, S., Gjorgovska, N., Kistanova, E. (2019). Dose-dependent effect of tribulus terrestris dry extract on reproductive organs of growing male rabbits.

Abarikwu, S.O., Onuah, C.L., & Singh, S.K. (2020). Plants in the management of male infertility. Andrologia, 52(3), e13509.

Abdel-All, S.R., Shakour, Z.T.A., Abouhussein, D.M., Reda, E., Sallam, T.F., El-Hefnawy, H.M., & Abdel-Monem, A.R. (2021). Phytochemical and biological evaluation of a newly designed nutraceutical self-nanoemulsifying self-nanosuspension for protection and treatment of cisplatin induced testicular toxicity in male rats. Molecules, 26(2), 408.

Abdul-Hamid, M., Abdel-Reheim, E.S., Hegazy, W., Allam, A.A., Othman, S.I., ALqhtani, H., & Abdel-Kawi, S.H. (2023). Impact of gervital against histopathological, ultrastructural, and biochemical alterations caused by methotrexate or azathioprine in albino rat testis. Environmental Science and Pollution Research, 30(8), 21914-21926.

Abreu, A.P., & Kaiser, U.B. (2022). Gonadotrophin Hormones. In The Pituitary (pp. 209-255). Academic Press.

Aebi, H. (1984). Catalase in vitro. In Methods in enzymology Academic press.

Aitken, R.J., & Drevet, J.R. (2020). The importance of oxidative stress in determining the functionality of mammalian spermatozoa: a two-edged sword. Antioxidants, 9(2), 111.

Aitken, R.J., & Lewis, S.E. (2023). DNA damage in testicular germ cells and spermatozoa. When and how is it induced? How should we measure it? What does it mean? Andrology, 11(8), 1545-1557.

Aitken, R.J., Drevet, J.R., Moazamian, A., & Gharagozloo, P. (2022). Male infertility and oxidative stress: a focus on the underlying mechanisms. Antioxidants, 11(2), 306.

Akalewold, M., Yohannes, G.W., Abdo, Z.A., Hailu, Y., & Negesse, A. (2022). Magnitude of infertility and associated factors among women attending selected public hospitals in Addis Ababa, Ethiopia: a cross-sectional study. BMC Women's Health, 22, 1-11.

Akbar, S. (2020). Handbook of 200 medicinal plants: a comprehensive review of their traditional medical uses and scientific justifications.

Akbar, S., & Akbar, S. (2020). Tribulus terrestris L.(Zygophyllaceae) (Syns.: T. lanuginosus L.; T. saharae A. Chev.). Handbook of 200 Medicinal Plants: A Comprehensive Review of Their Traditional Medical Uses and Scientific Justifications, 1835-1850.

Akbary, M.F., Ariyo, T., & Jiang, Q. (2022). Sociocultural determinants of attitudes toward domestic violence among women and men in Afghanistan: Evidence from Afghanistan Demographic and Health Survey 2015. Journal of interpersonal violence, 37(11-12).

Akbary, P., Daliran, S., Amini Khoei, Z. (2020). Effect of methanol extract of gokshura (Tribulus terrestris) on liver and digestive enzymes and blood biochemical parameters in grey mullet, Mugil cephalus (Linnaeus 1758). Journal of Aquaculture Development.;13(4):13-25.

Alahmadi, B.A. (2020). Effect of herbal medicine on fertility potential in experimental animals-An update review. Materia socio-medica, 32(2), 140.

Alahmar, A. T. (2019). Role of oxidative stress in male infertility: an updated review. Journal of human reproductive sciences, 12(1), 4-18.

Alam, A., Farooq, U., Singh, R., Dubey, V. P., Kumar, S., Kumari, R. & Dhar, K. L. (2018). Chemotherapy treatment and strategy schemes: A review. Open Access Journal of Toxicology, 2(5).

Alam, F., Khan, R., & Syed, F. (2024). Introduction to oxidative stress. In Fundamental Principles of Oxidative Stress in Metabolism and Reproduction (pp. 3-16). Academic Press.

Al-Eisa, R.A., Tag, H.M., El-Naggar, M.S., Abdelrazek, H., El-Shenawy, N.S. (2022). Evaluation of Tribulus terrestris extracts relative to metformin on oxidative stress and histopathology of the liver for diabetic male rats. Diabetology.;3(1):46-55.

Alemany, M. (2022). The roles of androgens in humans: biology, metabolic regulation and health. International journal of molecular sciences, 23(19), 11952.

Alexander, P., Kucera, G., & Pardee, T.S. (2016). Improving nucleoside analogs via lipid conjugation: Is fatter any better?. Critical reviews in oncology/hematology, 100, 46-56.

Alharbi, F. (2024). Ameliorative effects of L-carnitine as an antioxidant against testicular toxicity induced by AlCl 3 in male albino rats. European Review for Medical & Pharmacological Sciences, 28(5).

Ali, S.I., Gaafar, A.A., Abdallah, A.A., El-Daly, S.M., El-Bana, M., & Hussein, J. (2018). Mitigation of Alpha-Cypermethrin-Induced Hepatotoxicity in Rats by Tribulus terrestris Rich in Antioxidant Compounds. Jordan Journal of Biological Sciences, 11(5).

Allen, C.M., Lopes, F., Mitchell, R.T., Spears, N. (2018). How does chemotherapy treatment damage the prepubertal testis? Reproduction.;156(6).

Almasi, F., Khazaei, M., Chehrei, S., Ghanbari, A. (2017). Hepatoprotective effects of Tribulus terrestris hydroalcholic extract on non-alcoholic fatty liver-induced rats. Int J Morphol.;35(1):345-350.

Amin, A., Lotfy, A.M., Shafiullah, A.M., and Adeghate, E. (2006). The protective effect of Tribulus terrestris in diabetes. Ann N Y Acad Sci., 1084: 391-401.

Anand, U., Dey, A., Chandel, A. K. S., Sanyal, R., Mishra, A., Pandey, D. K. & de la Lastra, J. M. P. (2024). Corrigendum to 'Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics'[Genes & diseases]. Genes & Diseases, 11(4).

Anso, A.H. (2023). Hormones FSH, LH and Inhibin b levels in adolescent treated and followed due to varicocele at pediatric surgery Split. University of Split. School of Medicine.

Ara, C., Arshad, A., Faheem, M., Khan, M., & Shakir, H. A. (2022). Protective Potential of Aqueous Extract of Allium cepa against Tartrazine Induced Reproductive Toxicity. Pakistan veterinary journal, 42(3).

Arwanih, E.Y., Louisa, M., Rinaldi, I., & Wanandi, S.I. (2022). Resistance mechanism of acute myeloid leukemia cells against daunorubicin and cytarabine: a literature review. Cureus, 14(12).

Asadi, N., Bahmani, M., Kheradmand, A., RafieianKopaei, M. (2017). The impact of oxidative stress on testicular function and the role of antioxidants in improving it: a review. J Clin Diagn Res.

Ayman, A.H., Anas, A.O., Yasser, A.A., Masad, A.M., Abdullah, A.S., Mazen, A., & Al-Gthami, O. (2021). Levels of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone and Prolactin at Moderate Altitude Inhabitant Male. Pakistan Journal of Biological Sciences: PJBS, 24(2), 188-192.

Azam, F., Munier, S., Batoo, M., Ahmad, B., & Abbas, G. (2019). A review on advancements in ethnomedicine and phytochemistry of Tribulus terrestris–a plant with multiple health benefits. International Journal of Biosciences, 14(1), 21-37.

Bameri, B., Armandeh, M., Haghi-Aminjan, H., Rahimifard, M., Hassani, S., Gholami, M., & Abdollahi, M. (2024). Insights into the role of melatonin on the reproductive system toxicity induced by bisphenol A: a systematic review of preclinical studies. Toxin Reviews, 1-20.

Barbosa, M. L., de Meneses, A. A. P. M., de Aguiar, R. P. S., e Sousa, J. M. D. C., Cavalcante, A. A. D. C. M., & Maluf, S. W. (2020). Oxidative stress, antioxidant defense and depressive disorders: a systematic review of biochemical and molecular markers. Neurology, Psychiatry and Brain Research, 36, 65-72.

Bejarano, I., Espino, J., Paredes, S. D., Ortiz, A., Lozano, G., Pariente, J. A., & Rodríguez, A. B. (2012). Apoptosis, ROS and calcium signaling in human spermatozoa: relationship to infertility. Male Infertility, 53-76.

Bergmeyer, H.U., (1976). "Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes Part 2. IFCC method for aspartate aminotransferase." Clinica Chimica Acta 70.2 (1976).

Bhakta, S., & Das, S.K. (2020). The medicinal values of Abrus precatorius: a review study. J Adv Biotechnol Exp Ther, 3(2), 84-91.

Bhattacharya, I., Sharma, S.S., & Majumdar, S.S. (2024). Etiology of male infertility: an update. Reproductive Sciences, 31(4), 942-965.

Bisht, S., & Dada, R.J.F.B. (2017). Oxidative stress: Major executioner in disease pathology, role in sperm DNA damage and preventive strategies. Front Biosci (Schol Ed), 9(3), 420-47.

Biyik, A.F., Yulug, E., Yenilmez, E., Kutlu, A., Alver, A., & Erdem, S. (2024). Effects of quercetin on gentamicin-induced experimental testicular injury in rats. Romanian Journal of Morphology and Embryology= Revue Roumaine de Morphologie et Embryologie, 65(1), 69-80.

Boddu, P.C., Senapati, J., Ravandi-Kashani, F., Jabbour, E.J., Jain, N., Ayres, M., & Kadia, T. M. (2023). A phase 1 study to evaluate the safety, pharmacology, and feasibility of continuous infusion nelarabine in patients with relapsed and/or refractory lymphoid malignancies. Cancer, 129(4), 580-589.

Boota, M., Shah, S. M. A., Rashid, A., Akram, M., Ayaz, S., Mustafa, I., & Nisar, Z. (2022). The Hepatoprotective and Anti-Nephrotoxic Potential of Methanolic Extract of a Polyherbal Preparation in CCl4-Induced Liver Injury Model of Wistar Rats. Dose-Response, 20(3).

Bouabdallah, S., Ben-Attia, M., & Eldahshan, O.A. (2024). Efficacy of Tribulus terrestris on diabetes and sexual disorders: A narrative review. Journal of Bioresources and Environmental Sciences, 3(3), 155-160.

Bui, A.D., Sharma, R., Henkel, R., & Agarwal, A. (2018). Reactive oxygen species impact on sperm DNA and its role in male infertility. Andrologia, 50(8).

Chao, H.H., Zhang, Y., Dong, P.Y., Gurunathan, S., & Zhang, X.F. (2023). Comprehensive review on the positive and negative effects of various important regulators on male spermatogenesis and fertility. Frontiers in Nutrition, 9.

Chen, C., Hao, X., Lai, X., Liu, L., Zhu, J., Shao, H., & Zheng, J. (2021). Oxidative phosphorylation enhances the leukemogenic capacity and resistance to chemotherapy of B cell acute lymphoblastic leukemia. Science advances, 7(11).

Chen, J., & Ding, Z. (2024). Natural products as potential drug treatments for acute promyelocytic leukemia. Chinese Medicine, 19(1), 57.

Cheng, C.Y., & Mruk, D. D. (2010). The biology of spermatogenesis: the past, present and future. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1546), 1459-1463.

Chhatre, S., Nesari, T., Somani, G., Kanchan, D., Sathaye, S. (2014). Phytopharmacological overview of Tribulus terrestris. Pharmacogn Rev. ;8(15):45.

Chiou, J.T., Hsu, C.C., Hong, Y.C., Lee, Y.C., & Chang, L.S. (2023). Cytarabine-induced destabilization of MCL1 mRNA and protein triggers apoptosis in leukemia cells. Biochemical Pharmacology, 211, 115494.

Chocu, S., Calvel, P., Rolland, A.D., & Pineau, C. (2012). Spermatogenesis in mammals: proteomic insights. Systems biology in reproductive medicine, 58(4), 179-190.

Corpuz-Hilsabeck, M., & Culty, M. (2023). Impact of endocrine disrupting chemicals and pharmaceuticals on Sertoli cell development and functions. Frontiers in Endocrinology, 14, 1095894.

Cremer, T., Cremer, M., Hübner, B., Silahtaroglu, A., Hendzel, M., Lanctôt, C., & Cremer, C. (2020). The interchromatin compartment participates in the structural and functional organization of the cell nucleus. BioEssays, 42(2), 1900132.

Da Silva, M.H.A., Buys-Gonçalves, G.F., Estrada, J.H.D.R., Sampaio, F.J.B., de Souza, D.B. (2023). What is the effect of Tribulus terrestris on testicular morphology and sperm production? An experimental study in the rat model. Asian Journal of Andrology.:10.4103.

Da Silva, R., & Casella, T. (2022). Healthcare-associated infections in patients who are immunosuppressed due to chemotherapy treatment: a narrative review. The Journal of Infection in Developing Countries, 16(12), 1784-1795.

Das, P. K., Mukherjee, J., & Banerjee, D. (2023). Spermatogenesis and Semen. In Textbook of Veterinary Physiology. Singapore: Springer Nature Singapore.

Datta, S., Aggarwal, D., Sehrawat, N., Yadav, M., Sharma, V., Sharma, A., & Wang, H. (2023). Hepatoprotective effects of natural drugs: Current trends, scope, relevance and future perspectives. Phytomedicine.

De Luca, M.N., Colone, M., Gambioli, R., Stringaro, A., & Unfer, V. (2021). Oxidative stress and male fertility: role of antioxidants and inositols. Antioxidants, 10(8), 1283.

De Pascali, F., Tréfier, A., Landomiel, F., Bozon, V., Bruneau, G., Yvinec, R. & Reiter, E. (2018). Follicle-stimulating hormone receptor: advances and remaining challenges. International review of cell and molecular biology, 338, 1-58.

Delessard, M., Saulnier, J., Rives, A., Dumont, L., Rondanino, C., & Rives, N. (2020). Exposure to chemotherapy during childhood or adulthood and consequences on spermatogenesis and male fertility. International journal of molecular sciences, 21(4), 1454.

Di Francia, R.; Crisci, S.; De Monaco, A.; Cafiero, C.; Re, A.; Iaccarino, G.; De Filippi, R.; Frigeri, F.; Corazzelli, G.; Micera, A. (2021). Response and Toxicity to Cytarabine Therapy in Leukemia and Lymphoma: From Dose Puzzle to Pharmacogenomic Biomarkers. Cancers, 13, 966.

Doval, Y., Vilares, J., & Gómez-Rodríguez, C. (2020). Towards robust word embeddings for noisy texts. Applied Sciences, 10(19), 6893.

Drenberg, C.D., Hu, S., Li, L., Buelow, D.R., Orwick, S.J., Gibson, A.A., & Baker, S.D. (2016). ABCC4 is a determinant of cytarabine-induced cytotoxicity and myelosuppression. Clinical and translational science, 9(1), 51-59.

Drevet, J.R., Hallak, J., Nasr-Esfahani, M.H., & Aitken, R.J. (2022). Reactive oxygen species and their consequences on the structure and function of mammalian spermatozoa. Antioxidants & Redox Signaling, 37(7-9), 481-500.

Du, C., Yu, Y., & Fan, X. (2024). Analysis of research trends (2014-2023) on oxidative stress and male fertility based on bibliometrics and knowledge graphs. Frontiers in Endocrinology, 15.

Duy, C., Li, M., Teater, M., Meydan, C., Garrett-Bakelman, F.E., Lee, T.C., & Melnick, A. M. (2021). Chemotherapy induces senescence-like resilient cells capable of initiating AML recurrence. Cancer discovery, 11(6), 1542-1561.

Eleawa, S.M., Alkhateeb, M.A., Alhashem, F.H., Bin-Jaliah, I., Sakr, H.F., Elrefaey, H.M., & Khalil, M.A. (2014). Resveratrol reverses cadmium chloride-induced testicular damage and subfertility by downregulating p53 and Bax and upregulating gonadotropins and Bcl-2 gene expression. Journal of reproduction and development, 60(2), 115-127.

EL-Nashar, H.A., Kocaeli, S., Abdallah, M. & EL-shazly, M. (2023). Drug from Marine Sampling to Factory. Marine Ecosystems: A Unique Source of Valuable Bioactive Compounds, 3, 355-393.

El-Sakka, A. I. (2018). Dehydroepiandrosterone and erectile function: a review. The world journal of men's health, 36(3), 183.

El-Tohamy, M. M. (2012). The mechanisms by which oxidative stress and free radical damage produces male infertility. Life Sci J, 9(1), 674-688.

Endo, T., Freinkman, E., de Rooij, D.G., & Page, D.C. (2017). Periodic production of retinoic acid by meiotic and somatic cells coordinates four transitions in mouse spermatogenesis. Proceedings of the National Academy of Sciences, 114(47), E10132-E10141.

Esmaeilzadeh, A.A., & Nasirzadeh, F. (2022). Investigation of Chemicals on Breast Cancer. Eurasian Journal of Chemical, Medicinal and Petroleum Research, 1(5), 51-75.

Essawy, A., Matar, S., Mohamed, N., Abdel-Wahab, W., & Abdou, H. (2024). Ginkgo biloba extract protects against tartrazine-induced testicular toxicity in rats: involvement of antioxidant, anti-inflammatory, and anti-apoptotic mechanisms. Environmental Science and Pollution Research, 31(10), 15065-15077.

Esteves, S.C., Miyaoka, R., & Agarwal, A. (2011). An update on the clinical assessment of the infertile male. Clinics, 66(4), 691-700.

Eugeni, E., Arato, I., Del Sordo, R., Sidoni, A., Garolla, A., Ferlin, A. & Luca, G. (2022). Fertility preservation and restoration options for pre-pubertal male cancer patients: current approaches. Frontiers in Endocrinology, 13, 877537.

Fajardo-Orduña, G. R., Ledesma-Martínez, E., Aguiñiga-Sánchez, I., Mora-García, M. D. L., Weiss-Steider, B., & Santiago-Osorio, E. (2021). Inhibitors of chemoresistance pathways in combination with Ara-C to overcome multidrug resistance in AML. A mini review. International Journal of Molecular Sciences, 22(9), 4955.

Fang, Y., & Zhong, R. (2020). Effects of oxidative stress on spermatozoa and male infertility. Free Radic. Med. Biol, 10.

Faruqi, A. & Tadi, P. (2023). Cytarabine. In StatPearls. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK557680

Fatima, L., Sultana, A., Ahmed, S. and Sultana, S. (2015). Pharmacological activities of Tribulus terrestris Linn: a systemic review. World J Pharm Pharm Sci., 4: 136-150.

Foghis, M., Bungau, S.G., Bungau, A.F., Vesa, C.M., Purza, A.L., Tarce, A.G., & Radu, A.F. (2023). Plants-based medicine implication in the evolution of chronic liver diseases. Biomedicine & Pharmacotherapy, 158, 114207.

Gałęzowska, G., Ratajczyk, J., & Wolska, L. (2021). Determination of amino acids in human biological fluids by high-performance liquid chromatography: Critical review. Amino Acids, 53(7), 993-1009.

Gauthaman, K., Ganesan, A.P. (2006). The hormonal effects of Tribulus terrestris and its role in the management of male erectile dysfunction: an evaluation using primates, rabbit and rat. Phytomedicine. 2006; 15(3): 44-54.

Gavas, S., Quazi, S., & Karpiński, T.M. (2021). Nanoparticles for cancer therapy: current progress and challenges. Nanoscale research letters, 16(1), 173.

Ghafouri-Fard, S., Shoorei, H., Abak, A., Seify, M., Mohaqiq, M., Keshmir, F. (2021). Effects of chemotherapeutic agents on male germ cells and possible ameliorating impact of antioxidants. Biomedicine & Pharmacotherapy. ;142:112040.

Gilleland Marchak, J., Seidel, K.D., Mertens, A.C., Ritenour, C.W., Wasilewski-Masker, K., Leisenring, W.M., & Meacham, L.R. (2018). Perceptions of risk of infertility among male survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. Cancer, 124(11), 2447-2455.

Giwercman, A., & Petersen, P.M. (2000). Cancer and male infertility. Best Practice & Research Clinical Endocrinology & Metabolism, 14(3), 453-471.

Goswami, P., Damor, S., & Sharma, U.K. (2021). Effect of steroidal extract of Tribulus alatus on semen parameters of albino mice. Tobacco Regulatory Science, 7(6-2), 58-62.

Grewal, P.K. (2014). Multimodality based Tissue Classification Technique for Malignant Anomaly Detection.

Guo, Y., Shi, D.Z., Yin, H.J., Chen, K.J. (2007). Effects of Tribuli saponins on ventricular remodeling after myocardial infarction in hyperlipidemic rats. Am J Chin Med 2007; 35:309-16.

Gupta, R., Rajpoot, K., Tekade, M., Sharma, M.C., Safavi, M., & Tekade, R. K. (2022). Factors influencing drug toxicity. In Pharmacokinetics and Toxicokinetic Considerations (pp. 27-50). Academic Press.

Gurnari, C., Pagliuca, S., & Visconte, V. (2020). Deciphering the therapeutic resistance in acute myeloid leukemia. International journal of molecular sciences, 21(22), 8505.

Habib, R., Wahdan, S.A., Gad, A.M., & Azab, S.S. (2019). Infliximab abrogates cadmium-induced testicular damage and spermiotoxicity via enhancement of steroidogenesis and suppression of inflammation and apoptosis mediators. Ecotoxicology and environmental safety, 182, 109398.

Hadwan, M.H.; & Abed, H.N. (2016). Data supporting the spectrophotometric method for the estimation of catalase activity. Data in brief, 6, 194-199.

Hammoda, H.M., Ghazy, N.M., Harraz, F.M., Radwan, M.M., El Sohly, M.A., and Abdallah, I.I. (2013). Chemical constituents from Tribulus terrestris and screening of their antioxidant activity. Phytochem., 92: 153-159.

Hamzah, R.K., AL-Musawi, J.E.Q. (2023). Histological Study of Alteration in Testes and Epididymis of Domestic Rabbits Caused by Tribulus Terrestris

and Vitamin E. Archives of Razi Institute. 2023;78(4):1239-46. DOI: 10.32592/ARI.2023.78.4.1239

Han, J., Choi, S., Hong, J., Gang, D., Lee, S., Shin, K. & Kim, S.H. (2024). Superoxide Dismutase-Mimetic Polyphenol-Based Carbon Dots for Multimodal Bioimaging and Treatment of Atopic Dermatitis. ACS Applied Materials & Interfaces.

Han, S., Xiu, M., Li, S., Shi, Y., Wang, X., Lin, X., & He, J. (2023). Exposure to cytarabine causes side effects on adult development and physiology and induces intestinal damage via apoptosis in Drosophila. Biomedicine & Pharmacotherapy, 159, 114265.

Harrison, R. & Stringer, S. (1990). Use of fluorescent probes to assess membrane integrity in mammalian spermatozoa. Journal of reproduction and fertility. 88. 343-52. 10.1530/jrf.0.0880343.

Hong, Y., Boiti, A., Vallone, D., & Foulkes, N.S. (2024). Reactive Oxygen Species Signaling and Oxidative Stress: Transcriptional Regulation and Evolution. Antioxidants, 13(3), 312.

Houda, A., Nyaz, S., Sobhy, B.M., Bosilah, A.H., Romeo, M., Michael, J.P., & Eid, H.M. (2021). Seminiferous tubules and spermatogenesis. Male Reproductive Anatomy.

Houshmand, M., Garello, F., Circosta, P., Stefania, R., Aime, S., Saglio, G., & Giachino, C. (2020). Nanocarriers as magic bullets in the treatment of leukemia. Nanomaterials, 10(2), 276.

Howell, S.J. and Shalet, S.M. (2001). Testicular function following chemotherapy. European society of human reproduction and embryology. 7,4:363-369.

Howland, R.H., Rush, A.J., Wisniewski, S.R., Trivedi, M.H., Warden, D., Fava, M. & Berman, S.R. (2009). Concurrent anxiety and substance use disorders

among outpatients with major depression: clinical features and effect on treatment outcome. Drug and alcohol dependence, 99(1-3), 248-260.

Hussain, J., Khan, A.L., Rehman, N., Hamayun, M., Shah, T., Nisar, Bano, T., Shinwari, Z.K., Lee, I.J. (2009). Proximate and nutrient analysis of selected vegetable species: A case study of Karak Region Pakistan. Afr. J. Biotechnol. 8(12): 2725-2729.

Hussain, T., Kandeel, M., Metwally, E., Murtaza, G., Kalhoro, D.H., Yin, Y., & Kalhoro, M.S. (2023). Unraveling the harmful effect of oxidative stress on male fertility: A mechanistic insight. Frontiers in Endocrinology, 14, 1070692.

Hussain, T., Metwally, E., Murtaza, G., Kalhoro, D.H., Chughtai, M.I., Tan, B., & Kalhoro, M. S. (2024). Redox mechanisms of environmental toxicants on male reproductive function. Frontiers in Cell and Developmental Biology, 12, 1333845.

Iancu, M.E., Albu, A.I., & Albu, D.N. (2023). Prolactin Relationship with Fertility and In Vitro Fertilization Outcomes—A Review of the Literature. Pharmaceuticals, 16(1), 122.

Ibrahim, N.A., Al-Shmgani, H.S., & Ibrahim, R. (2017). Cytarabine induced reproductive histopathological changes in albino male mice. Journal of Biotechnology Research Center, 11(1), 6-12.

Ibtisham, F., Wu, J., Xiao, M., An, L., Banker, Z., Nawab, A., & Li, G. (2017). Progress and future prospect of in vitro spermatogenesis. Oncotarget, 8(39), 66709.

Ilacqua, A., Francomano, D., & Aversa, A. (2018). The physiology of the testis. Principles of Endocrinology and Hormone Action, Endocrinology. Switzerland: Springer International Publishing AG, 1-38.

Inwati, P., Shakya, N., Chouksey, S., Bisen, A., Kumar, J., & Aharwal, B. (2022). Effect of herbs to protect oxidative stress in sperm and male fertility: a review. Pharm Innov J, 11(5), 2550-2554.

Ivanova, A., Lazarova, I., Mechkarova, P., Semerdjieva, I., and Evstatieva, L. (2011). Intraspecific variability of biologically active compounds of different populations of Tribulus terrestris in Thracian floristic region. Biotechnol Biotechnol Equip., 25: 2357-2361.

James, E.R., Carrell, D.T., Aston, K.I., Jenkins, T.G., Yeste, M., Salas-Huetos, A. (2020). The role of the epididymis and the contribution of epididymosomes to mammalian reproduction. Int J Mol Sci.;21(15):5377.

Jana, K., Jana, N., De, D.K., & Guha, S.K. (2010). Ethanol induces mouse spermatogenic cell apoptosis in vivo through over-expression of Fas/Fas-L, p53, and caspase-3 along with cytochrome c translocation and glutathione depletion. Molecular reproduction and development, 77(9), 820-833.

Jarabak, J., Adams, J.A., Williams-Ashman, H.G., Talalay, P. (1962). Purification of a 17β-Hydroxysteroid Dehydrogenase of Human Placenta and Studies on Its Transhydrogenase Function, Journal of Biological Chemistry, Volume 237, Issue 2.

Jaradat, N., & Zaid, A.N. (2019). Herbal remedies used for the treatment of infertility in males and females by traditional healers in the rural areas of the West Bank/Palestine. BMC Complementary and Alternative Medicine, 19, 1-12.

Jarić, S., Kostić, O., Mataruga, Z., Pavlović, D., Pavlović, M., Mitrović, M., & Pavlović, P. (2018). Traditional wound-healing plants used in the Balkan region (Southeast Europe). Journal of ethnopharmacology, 211, 311-328.

Jomova, K., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2024). Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. Archives of Toxicology, 1-45.

Karimi, J., Malekzadeh, H., Shiravani, S. (2012). Effect of the tribulus terrestris extract on spermatogenesis in the rat. Journal of Jahrom University of Medical Sciences.; 9(4): 7-11.

Katami, H., Suzuki, S., Fujii, T., Ueno, M., Tanaka, A., Ohta, K. I., & Shimono, R. (2023). Genetic and histopathological analysis of spermatogenesis after short-term testicular torsion in rats. Pediatric Research, 94(5), 1650-1658.

Kavsak, P.A., Worster, A., Hill, S.A., MacRae, A.R., & Jaffe, A.S. (2017). Analytical comparison of three different versions of a high-sensitivity cardiac troponin I assay over 10 years. Clinica Chimica Acta, 475, 51-55.

Kesari, K.K., Agarwal, A., & Henkel, R. (2018). Radiations and male fertility. Reproductive Biology and Endocrinology, 16(1), 118.

Keshtmand, Z., Ghanbari, A., & Keshtmand, R. (2014). Protective Effect of Tribulus Terrestris Hydroalcoholic Extract against Cisplatin–Induce Apoptosis on Testis in Mice. Anatomical Sciences Journal, 11(2), 75-80.

Khadim, S., Malik, K., Qureshi, R., & Rehman, S. (2023). Ethnogynecological study of traditional therapeutic plants used by the indigenous communities: A case study from District Gujrat Punjab, Pakistan. Ethnobotany Research and Applications, 26, 1-23.

Khaleel, B., Lunenfeld, E., Kapelushnik, J., Huleihel, M. (2022). Effect of Chemotherapy Cytarabine and Acute Myeloid Leukemia on the Development

of Spermatogenesis at the Adult Age of Immature Treated Mice. International Journal of Molecular Sciences.;23(7):4013.

Khaleghi, S., Bakhtiari, M., Asadmobini, A., & Esmaeili, F. (2017). Tribulus terrestris extract improves human sperm parameters in vitro. Journal of evidence-based complementary & alternative medicine, 22(3), 407-412.

Khan, M.N., Mallela, A., Nishanthi, M.G., & Chandran, L. (2023). Pre-clinical and Clinical Evidence Associated with Infertility in Men: Future Aspects. Current Chinese Science, 3(4), 244-252.

Khokhlova, E.V., Fesenko, Z.S., Sopova, J.V., & Leonova, E.I. (2020). Features of DNA repair in the early stages of mammalian embryonic development. Genes, 11(10), 1138.

Kiss, R.C., Xia, F., & Acklin, S. (2021). Targeting DNA damage response and repair to enhance therapeutic index in cisplatin-based cancer treatment. International journal of molecular sciences, 22(15), 8199.

Kolure, R., Nachammai, V., Manjula, S.N., Godela, R., Bhavani, D.S., & Rajendra, Y. (2023). Hepatoprotective effect of Swertiamarin from Cytarabine Induced Hepatotoxicity in Pregnant rats. Research Journal of Pharmacy and Technology, 16(12), 5707-5712.

Konopleva, M.Y., Röllig, C.,Cavenagh, J., Deeren, D., Girshova, L., Krauter, J., & Wei, A.H. (2022). Idasanutlin plus cytarabine in relapsed or refractory acute myeloid leukemia: results of the MIRROS trial. Blood Advances, 6(14), 4147-4156.

Koppers, A.J., Mitchell, L.A., Wang, P., Lin, M., & Aitken, R.J. (2011). Phosphoinositide 3-kinase signalling pathway involvement in a truncated apoptotic cascade associated with motility loss and oxidative DNA damage in human spermatozoa. Biochemical Journal, 436(3), 687-698.

Kostova, I., and Dinchev, D. (2005). Saponins in Tribulus terrestris - chemistry and bioactivity. Phytochem Rev., 4: 111-137.

Krishnamurthy, H.K., Pereira, M., Jayaraman, V., Krishna, K., Wang, T., Bei, K., & Rajasekaran, J.J. (2024). Inside the Genome: Understanding Genetic Influences on Oxidative Stress.

Kubota, H., & Brinster, R.L. (2018). Spermatogonial stem cells. Biology of reproduction, 99(1), 52-74.

Kumar, N., & Singh, A. K. (2022). Impact of environmental factors on human semen quality and male fertility: a narrative review. Environmental Sciences Europe, 34, 1-13.

Kumar, N., Singh, A.K. (2015). Trends of male factor infertility, an important cause of infertility: A review of literature. J Hum Reprod Sci. 8(4):191. doi: 10.4103/0974-1208.170370.

Kutteh, W.H., Stanic, A.K., & Schust, D.J. (2019). Immunology and reproduction. In Yen and Jaffe's Reproductive Endocrinology (pp. 301-321). Elsevier.

Lala, V., Zubair, M., & Minter, D. (2023). Liver function tests. StatPearls.

Lamirande, E.D., & Gagnon, C. (1993). A positive role for the superoxide anion in triggering hyperactivation and capacitation of human spermatozoa. International Journal of Andrology, 16(1), 21-25.

Lenzi, A., Picardo, M., Gandini, L., & Dondero, F. (1996). Lipids of the sperm plasma membrane: from polyunsaturated fatty acids considered as markers of sperm function to possible scavenger therapy. Human reproduction update, 2(3), 246-256.

Leslie, S., Soon-Sutton, T., & Khan, M. A. (2024). Male infertility. StatPearls.

Li, S., Zhao, S., Guo, Y., Yang, Y., Huang, J., Wang, J., & Chin, Y. (2022). Clinical efficacy and potential mechanisms of acupoint stimulation combined with

chemotherapy in combating cancer: A review and prospects. Frontiers in Oncology, 12, 864046. Li et al., 2022

Li, Z., Guo, J.R., Chen, Q.Q., Wang, C.Y., Zhang, W.J., Yao, M.C., & Zhang, W. (2017). Exploring the antitumor mechanism of high-dose cytarabine through the metabolic perturbations of ribonucleotide and deoxyribonucleotide in human promyelocytic leukemia HL-60 cells. Molecules, 22(3), 499.

Liu, C., Yue, S., Solarz, J., Lee, J., Li, L. (2021). Improving the sexual activity and reproduction of female zebrafish with high testosterone levels. Scientific Reports.;11(1):3822.

Liu, J.L., Sun, C., Yu, Q., Liang, Y.Z., Lin, S.S., & Tian, M. (2022). Chemoprotective effect of daphnetin against benzene induced leukemia cancer via alteration of CYP2E1.

Lombardo, A., & Nichols, E. (2009). Toxicity and anti-tumor effects of cytarabine and doxorubicin on acute myeloid leukemia. A major qualifying project report submitted to the Faculty of the Worcester polytechnic Institute, Biology and Biotechnology and Biochemistry.

Lopes, F., Tholeti, P., Adiga, S. K., Anderson, R. A., Mitchell, R. T., & Spears, N. (2021). Chemotherapy induced damage to spermatogonial stem cells in prepubertal mouse in vitro impairs long-term spermatogenesis. Toxicology Reports, 8, 114-123.

Lundy, S., & Vij, S.C. (2022). Male infertility. In Clinical Reproductive Medicine and Surgery: A Practical Guide (pp. 265-279). Cham: Springer International Publishing.

Ma, H., & Shieh, K.J. (2006). Cholesterol and human health. The Journal of American Science, 2(1), 46-50.

Majed, M.S. (2022). Hepatoprodective effect of L-carnitine against cytarabine induced hepatotoxic in male rats (Doctoral dissertation, University of Kerbala).

Malviya, N., Malviya, S., Jain, S., & Vyas, S. (2016). A review of the potential of medicinal plants in the management and treatment of male sexual dysfunction. Andrologia, 48(8), 880-893.

Mansour, H.A.E.H. (2023). Infertility diagnosis and management. Beni-Suef University Journal of Basic and Applied Sciences, 12(1), 81.

Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European journal of biochemistry, 47(3), 469-474.

Men, J., Zhang, L., Peng, R., Li, Y., Li, M., Wang, H., & Guo, J. (2023). Metformin Ameliorates 2.856 GHz Microwave-Radiation-Induced Reproductive Impairments in Male Rats via Inhibition of Oxidative Stress and Apoptosis. International Journal of Molecular Sciences, 24(15), 12250.

Micozzi, D., Carpi, F. M., Pucciarelli, S., Polzonetti, V., Polidori, P., Vilar, S., & Vincenzetti, S. (2014). Human cytidine deaminase: a biochemical characterization of its naturally occurring variants. International journal of biological macromolecules, 63, 64-74.

Miranda, D.R., Chaves, B.R., Vilela Soares, L., Konig, I.F. M., Pinto, J.T., Miranda, J.R., & Zangeronimo, M.G. (2022). Use of *Tribulus terrestris* and Lepidium meyenii extract in rats: reproductive, biochemical and body parameters. Andrologia, 54(4), e14358.

Miyaso, H., Ogawa, Y., & Itoh, M. (2022). Microenvironment for spermatogenesis and sperm maturation. Histochemistry and cell biology, 157(3), 273-285.

Mottola, F., Palmieri, I., Carannante, M., Barretta, A., Roychoudhury, S., & Rocco, L. (2024). Oxidative Stress Biomarkers in Male Infertility: Established Methodologies and Future Perspectives. Genes, 15(5), 539.

Mughal, T.I., & Schrieber, A. (2010). Principal long-term adverse effects of imatinib in patients with chronic myeloid leukemia in chronic phase. Biologics: Targets and Therapy, 315-323.

Mujika, I., Busso, T., Lacoste, L., Barale, F., Geyssant, A., & Chatard, J.C. (1996). Modeled responses to training and taper in competitive swimmers. Medicine and science in sports and exercise, 28(2), 251-258.

Muñoz, E., Fuentes, F., Felmer, R., Arias, M.E., & Yeste, M. (2024). Effects of Reactive Oxygen and Nitrogen Species on Male Fertility. Antioxidants & Redox Signaling.

Muslih, R.K., AL-Nimer, M.S., & AL-Zamely, O.M.Y. (2002). The level of malondialdehyde after activation with (H2O and CuSO4) and inhibition by disferoxamine myocardial infraction. National Journal of Chemistry, 5, 139-148.

Namoju, R.C., Khan, S., Patel, R. S., Shera, F.Y., Trivedi, P.P., Kushwaha, S., & Jena, G.B. (2014). Pre-pubertal exposure of cytarabine-induced testicular atrophy, impaired spermatogenesis and germ cell DNA damage in SD rats. Toxicology Mechanisms and Methods, 24(9), 703-712.

Namoju, R.; and Chilaka, N.K. (2021). Alpha-lipoic acid ameliorates cytarabine-induced developmental anomalies in rat fetus. Human & Experimental Toxicology, 40(5), 851-868.

Naseri, L., Akbaribazm, M., Khazaei, M. (2019). A review on therapeutic effects of Tribulus terrestris. J Med Plants.;18(72):1-22. doi:10.29252/jmp.4.72.1

Neumann, J., Š Libalova, K., Konvalina, P., Smetana, P., Vrabli, P., & Ksoch, M. (2024). Tribulus terrestris L.-a review. Journal of Central European Agriculture, 25(1), 194-208.

Nikmahzar, A., Khadivi, F., Abbasi, M., Mahdavinezhad, F., Abbasi, Y., & Daneshi, E. (2023). Testicular tissue vitrification: a promising strategy for male fertility preservation. Reproductive Sciences, 30(6), 1687-1700.

Niran, A.I., Hanady, S.A., Ruqaya, M.F. (2017). cytarabine induced reproductive histopathological changes in albino male mice.; 11(6): 6-11.

Nishi, R., Shigemi, H., Negoro, E., Okura, M., Hosono, N., & Yamauchi, T. (2020). Venetoclax and alvocidib are both cytotoxic to acute myeloid leukemia cells resistant to cytarabine and clofarabine. BMC cancer, 20, 1-13.

Okada, K., and Fujisawa, M. (2019). Recovery of spermatogenesis following cancer treatment with cytotoxic chemotherapy and radiotherapy. The world journal of men's health.;37(2):166-74.

Okwute, P.G., Oluwatunase, G.O., Mofolorunso, A.M., Asafa, O.O., Ogunbiyi, O.E., & Olalekan, S. (2023). Evaluation of Abrus precatorius on reproductive function of male Wistar rat. Anatomy Journal of Africa, 12(2), 2384-2392.

Palla, A., & Ahmed, W. (2024). Overview of prevention and management of oxidative stress. Fundamental Principles of Oxidative Stress in Metabolism and Reproduction, 243-276.

Pan, J., Liu, P., Yu, X., Zhang, Z., & Liu, J. (2024). The adverse role of endocrine disrupting chemicals in the reproductive system. Frontiers in Endocrinology, 14, 1324993.

Park, N., Sharma, C., Jung, U.J., Kim, S., Nam, Y., Kim, K. S., & Kim, S.R. (2023). Mesenchymal Stem Cell Transplantation Ameliorates Ara-C-Induced Motor

Deficits in a Mouse Model of Cerebellar Ataxia. Journal of Clinical Medicine, 12(5), 1756.

Pavin, N.F., Izaguirry, A.P., Soares, M.B., Spiazzi, C.C., Mendez, A.S.L., Leivas, F.G. (2018). Tribulus terrestris protects against male reproductive damage induced by cyclophosphamide in mice. Oxidative Medicine and Cellular Longevity. 1–9.

Poulopoulos, A., Papadopoulos, P., Andreadis, D. (2017). Chemotherapy: oral side effects and dental interventions-a review of the literature. Stomatological Disease and Science.;1:35-49.

Qi, Q., Yang, J., Li, S., Liu, J., Xu, D., Wang, G., & Pan, X. (2024). Melatonin alleviates oxidative stress damage in mouse testes induced by bisphenol A. Frontiers in Cell and Developmental Biology, 12, 1338828.

Qureshi, A., Naughton, D.P., & Petroczi, A. (2014). A Systematic Review on the Herbal Extract *Tribulus terrestris* and the Roots of its Putative Aphrodisiac and Performance Enhancing Effect. Journal of Dietary Supplements, 11(1), 64–79. https://doi.org/10.3109/19390211.2014.887602

Rahimi, A., Asadi, F., Rezghi, M., Kazemi, S., Soorani, F., Memariani, Z. (2022). Natural products against cisplatin-induced male reproductive toxicity: A comprehensive review. Journal of Biochemical and Molecular Toxicology. ;36(3): e22970.

Rahmathulla, S.M., Maruthi, E., Bheemewsaraiah, K., Manjunatha, S., & Devi, K.L. (2012). Effect of Tribulus terrestris (L.) on liver in isoproterenol-induced myocardial infarction. Int J Res Biochem Biophys2012, 2(4), 10-12.

Raj, S., Khurana, S., Choudhari, R., Kesari, K.K., Kamal, M.A., Garg, N., & Kumar, D. (2021). Specific targeting cancer cells with nanoparticles and drug delivery

in cancer therapy. In Seminars in cancer biology (Vol. 69, pp. 166-177). Academic Press.

Ramaswamy, S., & Weinbauer, G.F. (2014). Endocrine control of spermatogenesis: Role of FSH and LH/testosterone. Spermatogenesis, 4(2), e996025.

Ramgir, S.S., Gopalakrishnan, A.V., & Roopan, S.M. (2021). Herbal Traditional Remedies for Male Infertility. Phytopharmaceuticals: Potential Therapeutic Applications, 173-189.

Rani, J., Dhull, S.B., Rose, P.K., & Kidwai, M.K. (2024). Drug-induced liver injury and anti-hepatotoxic effect of herbal compounds: a metabolic mechanism perspective. Phytomedicine, 122, 155142.

Rechkoblit, O., Johnson, R.E., Buku, A., Prakash, L., Prakash, S., & Aggarwal, A.K. (2019). Structural insights into mutagenicity of anticancer nucleoside analog cytarabine during replication by DNA polymerase η. Scientific reports, 9(1), 16400.

Ribeiro, J.C., Nogueira-Ferreira, R., Amado, F., Alves, M.G., Ferreira, R., & Oliveira, P.F. (2022). Exploring the role of oxidative stress in sperm motility: a proteomic network approach. Antioxidants & Redox Signaling, 37(7-9), 501-520.

Rjeibi, I., Saad, A.B., and Hfaiedh, N. (2016). Oxidative damage and hepatotoxicity associated with deltamethrin in rats: The protective effects of Amaranthus spinosus seed extract. Biomed Pharmacother., 84: 853-860.

Roaiah, M.F., Elkhayat, Y.I., GamalEl Din, S.F., & Abd El Salam, M.A. (2017). Prospective Analysis on the Effect of Botanical Medicine (Tribulus terrestris) on Serum Testosterone Level and Semen Parameters in Males with Unexplained Infertility. Journal of Dietary Supplements, 14(1), 25–31. https://doi.org/10.1080/19390211.2016.1188193

Rodriguez-Wallberg, K.A., Jiang, Y., Lekberg, T., & Nilsson, H.P. (2023). The late effects of cancer treatment on female fertility and the current status of fertility preservation—a narrative review. Life, 13(5), 1195.

Saad, A.A. (2022). Targeting cancer-associated glycans as a therapeutic strategy in leukemia. All Life, 15(1), 378-433.

Sadiq, I.Z. (2023). Free radicals and oxidative stress: signaling mechanisms, redox basis for human diseases, and cell cycle regulation. Current molecular medicine, 23(1), 13-35.

Safarinejad, M.R. (2008). Infertility among couples in a population-based study in Iran: prevalence and associated risk factors. International journal of andrology, 31(3), 303-314.

Saini, A., Kumar, M., Bhatt, S., Saini, V., & Malik, A. (2020). Cancer causes and treatments. Int. J. Pharm. Sci. Res, 11, 3121-3134.

Sakali, A.K., Bargiota, A., Papagianni, M., Rasic-Markovic, A., & Mastorakos, G. (2023). Environmental Impact on Female Fertility and Pregnancy. In Environmental Endocrinology and Endocrine Disruptors: Endocrine and Endocrine-targeted Actions and Related Human Diseases (pp. 1-27). Cham: Springer International Publishing.

Salas-Benito, D., Pérez-Gracia, J.L., Ponz-Sarvisé, M., Rodriguez-Ruiz, M.E., Martínez-Forero, I., Castañón, E., & Melero, I. (2021). Paradigms on immunotherapy combinations with chemotherapy. Cancer discovery, 11(6), 1353-1367.

Santos, H.O., Howell, S., & Teixeira, F.J. (2019). Beyond tribulus (*Tribulus terrestris* L.): The effects of phytotherapics on testosterone, sperm and prostate parameters. Journal of ethnopharmacology, 235, 392-405.

Saulnier, J., Chalmel, F., Delessard, M., Moutard, L., Pereira, T., Fraissinet, F., & Rives, N. (2022). Understanding the underlying molecular mechanisms of meiotic arrest during in vitro spermatogenesis in rat prepubertal testicular tissue. International Journal of Molecular Sciences, 23(11), 5893.

Schlebusch, H., Rick, W., Lang, H., & Knedel, M. (1974). Standards in the activities of clinically important enzymes. Deutsche medizinische Wochenschrift (1946), 99(15), 765-766.

Schumann, G.; Loth, E., Banaschewski, T.; Barbot, A., Barker, G.; Büchel, C.; & Struve, M. (2010). The IMAGEN study: reinforcement-related behaviour in normal brain function and psychopathology. Molecular psychiatry, 15(12), 1128-1139.

Seevola, D., Baebacini, G.M., Bona, S. (1984). Flavonoids and hepatic cyclic monophosphates in liver injury. Boll. Ins. Sieroter. Milan 63: 777–782; 1984.

Sengupta, P., Arafa, M., & Elbardisi, H. (2019). Hormonal regulation of spermatogenesis. In Molecular signaling in spermatogenesis and male infertility (pp. 41-49). CRC Press.

Sengupta, P., Dutta, S., & Irez, T. (2024). Oxidants and antioxidants in male reproduction: roles of oxidative and reductive stress. Journal of Integrated Science and Technology, 12(3), 753-753.

Sertoli, E. (2018). The structure of seminiferous tubules and the development of [spermatids] in rats. Biology of Reproduction, 99(3), 482-503.

Sharma, P., Kaushal, N., Saleth, L. R., Ghavami, S., Dhingra, S., & Kaur, P. (2023). Oxidative stress-induced apoptosis and autophagy: Balancing the contrary forces in spermatogenesis. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 166742.

Sharma, R., & Agarwal, A. (2018). Defective spermatogenesis and sperm DNA damage. A Clinician's Guide to Sperm DNA and Chromatin Damage, 229-261.

Shawish, M.I., Bagheri, B., Musini, V.M., Adams, S.P., & Wright, J.M. (2021). Effect of atorvastatin on testosterone levels. Cochrane Database of Systematic Reviews, (1).

Shiva, M., Gautam, A. K., Verma, Y., Shivgotra, V., Doshi, H., & Kumar, S. (2011). Association between sperm quality, oxidative stress, and seminal antioxidant activity. Clinical biochemistry.

Sigolo, R.P. (2022). Healing throug plants: relationships among knowledge systems in the Brazilian press. Herbs and the Evolution of Human Societies, 75.

Silva, A.F., Ramalho-Santos, J., & Amaral, S. (2021). The impact of antisperm antibodies on human male reproductive function: an update. Reproduction, 162(4), R55-R71.

Singh, A. (2023). Primate Spermatogonial Stem Cells and Their Niche: A Single-Cell View (Doctoral dissertation, The University of Texas at San Antonio).

Singh, J., Kaur, J., Kaur, M., Rana, A., Rasane, P., & Kaur, S. (2023). Tribulus terrestris: Pharmacological and Nutraceutical Potential. Harvesting Food from Weeds, 79-112.

Sirotkin, A.V., Kolesarova, A. (2021). Puncture Vine (Tribulus Terrestris L.) in Control of Health and Reproduction. Physiol Res.;70(4):S657.

Sriharikrishnaa, S., Suresh, P.S., & Prasada K,S. (2023). An Introduction to Fundamentals of Cancer Biology. In Optical Polarimetric Modalities for Biomedical Research (pp. 307-330). Cham: Springer International Publishing.

Ștefănescu, R., Farczadi, L., Huțanu, A., Ősz, B.E., Mărușteri, M., Negroiu, A., & Vari, C.E. (2021). Tribulus terrestris efficacy and safety concerns in diabetes and erectile dysfunction, assessed in an experimental model. Plants, 10(4), 744.

Sudhakaran, G., Kesavan, D., Kandaswamy, K., Guru, A., & Arockiaraj, J. (2024). Unravelling the epigenetic impact: Oxidative stress and its role in male infertility-associated sperm dysfunction. Reproductive Toxicology, 108531.

Talapatra, A. (2021). Spermatogenesis requires nuclear androgen receptors in somatic cells and testosterone binding to membrane receptors on germ cells in rat testis (Doctoral dissertation).

Tannock, I.F., Pond, G.R., & Booth, C.M. (2022). Biased evaluation in cancer drug trials—how use of progression-free survival as the primary end point can mislead. JAMA oncology, 8(5), 679-680.

Thakur, S., Kumar, V., Das, R., Sharma, V., & Mehta, D.K. (2024). Biomarkers of Hepatic toxicity: An Overview. Current Therapeutic Research, 100737.

Thilagavathi, R., Begum, S.S., Varatharaj, S.D., Balasubramaniam, A.K., George, J.S., & Selvam, C. (2023). Recent insights into the hepatoprotective potential of medicinal plants and plant-derived compounds. Phytotherapy Research, 37(5), 2102-2118.

Thumfart, K.M., & Mansuy, I.M. (2023). What are Sertoli cells? Historical, methodological, and functional aspects. Andrology.

Tiwari, P., Yadav, A., Kaushik, M., & Dada, R. (2024). Cancer risk and male Infertility: Unravelling predictive biomarkers and prognostic indicators. Clinica Chimica Acta, 119670.

Tkachenko, K., Frontasyeva, M., Vasilev, A., Avramov, L., Shi, L. (2020). Major and trace element content of Tribulus terrestris l. wildlife plants. Plants.; 9(12):1764.

Toprak, T., & Kulaksiz, D. (2024). Oxidative stress, varicocele, and disorders of male reproduction. In Fundamental Principles of Oxidative Stress in Metabolism and Reproduction (pp. 215-232). Academic Press.

Tsuda, M., Kitamasu, K., Kumagai, C., Sugiyama, K., Nakano, T., & Ide, H. (2020). Tyrosyl-DNA phosphodiesterase 2 (TDP2) repairs topoisomerase 1 DNA-protein crosslinks and 3′-blocking lesions in the absence of tyrosyl-DNA phosphodiesterase 1 (TDP1). DNA repair, 91, 102849.

Uniyal, S., Kumar, N., & Joshi, B. C. (2024). An Overview on Free Radicals and Role of Antioxidants in the Management of Cancer (Brief Review). Oriental Journal of Chemistry, 40(1).

Uotila, M., Ruoslahti, E., & Engvall, E. (1981). Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alpha-fetoprotein. Journal of immunological methods, 42(1), 11-15.

Wang, D., Wu, W., Callen, E., Pavani, R., Zolnerowich, N., Kodali, S. & Nussenzweig, A. (2022). Active DNA demethylation promotes cell fate specification and the DNA damage response. Science, 378(6623), 983-989.

Wang, H., Yuan, Q., Niu, M., Zhang, W., Wen, L., Fu, H., & He, Z. (2018). Transcriptional regulation of P63 on the apoptosis of male germ cells and three stages of spermatogenesis in mice. Cell death & disease, 9(2), 76.

Wang, Q., Deng, J., Jiang, Y., Wang, Z., Xia, B., & Chen, T. (2023). A medaka gonad-specific lncRNA may act as pri-miR-202 to regulate testicular endocrine homeostasis and spermatogenesis. Theriogenology.

Wang, Y., Xu, Y., Yang, L., Yang, Y., Guo, A. L., Han, X. J. & Chao, L. (2024). N-acetylcysteine alleviated tris (2-chloroisopropyl) phosphate-induced sperm motility decline and functional dysfunction in mice through reversing oxidative stress and DNA damage. Ecotoxicology and Environmental Safety, 271, 116000.

Wang, Z. (2022). Cell cycle progression and synchronization: An overview. Cell-Cycle Synchronization: Methods and Protocols, 3-23.

Wegener, T. and Fintelmann, V. (1999). Flavonoids and Bioactivity. Wein Med. Wochem. Schr., 149: 241-247.

Wistuba, J., Neuhaus, N., & Nieschlag, E. (2023). Physiology of Testicular Function. In Andrology: Male Reproductive Health and Dysfunction (pp. 15-54). Cham: Springer International Publishing.

Wu, T.S, Shi, L.S. and Kuo, S.C. (1999). Alkaloids and other constituents from Tribulus terrestris. Phytochem., 50: 1411-1415.

Wu, X., Jiao, Z., Zhang, J., Li, F., & Li, Y. (2023). Expression of TFRC helps to improve the antineoplastic effect of Ara-C on AML cells through a targeted delivery carrier. Journal of Nanobiotechnology, 21(1), 126.

Wu, Z., Wu, Z., Zou, Y., Guan, J., Wu, S., & Chen, D. (2022). A clinical report on high-dose cytarabine therapy for children with acute myeloid leukemia. Molecular and Clinical Oncology, 17(5), 1-6.

Xu, J.J., Zhang, W.C., Guo, Y.W., Chen, X.Y., & Zhang, Y.N. (2022). Metal nanoparticles as a promising technology in targeted cancer treatment. Drug Delivery, 29(1), 664-678.

Yadav, K.S., & Sawant, K.K. (2010). Modified nanoprecipitation method for preparation of cytarabine-loaded PLGA nanoparticles. Aaps Pharmscitech, 11, 1456-1465.

Yahya, R.A., Attia, A.M., Azab, A.E., Karema, E.l.M., Shkal, and Mona, A. Yehia (2022). Treatment of Male Albino Rats with 5-fluorouracil and/or Cyclophosphamide Caused Serious Alterations in Sexual Hormones, Histological Structure of the Testis, and Semen Quality. Archives of Clinical Investigation, 1(1).

Ye, R. J. et al. Interplay between male reproductive system dysfunction and the therapeutic effect of flavonoids. Fitoterapia 147, 104756 (2020).

Yeo, C.I., Ooi, K.K., & Tiekink, E. R. (2018). Gold-based medicine: a paradigm shift in anti-cancer therapy?. Molecules, 23(6), 1410.

Yu, P. L., Pu, H. F., Chen, S. Y., Wang, S. W. & Wang, P. S. Effects of catechin, epicatechin and epigallocatechin gallate on testosterone production in rat leydig cells. J. Cell. Biochem. 110, 333–342 (2010).

Yue, Z., She, R., Bao, H., Li, W., Wang, D., Zhu, J., & Yu, P. (2011). Exposure to 3-methyl-4-nitrophenol affects testicular morphology and induces spermatogenic cell apoptosis in immature male rats. Research in Veterinary Science, 91(2), 261-268.

Zahid, N., Batool, S., & Sohail, E. (2024). Oxidative stress, mechanisms, and subfertility in male. In Fundamental Principles of Oxidative Stress in Metabolism and Reproduction (pp. 201-214). Academic Press.

Zeidan, A. M., DeAngelo, D. J., Palmer, J., Seet, C. S., Tallman, M. S., Wei, X., & Stock, W. (2022). Phase 1 study of anti-CD47 monoclonal antibody CC-90002 in patients with relapsed/refractory acute myeloid leukemia and high-risk myelodysplastic syndromes. Annals of hematology, 101(3), 557-569.

Zhang, M., Chen, X., & Radacsi, N. (2021). New tricks of old drugs: Repurposing non-chemo drugs and dietary phytochemicals as adjuvants in anti-tumor therapies. Journal of controlled release, 329, 96-120.

Zhang, Q., Kang, R., Zeh, III, H.J., Lotze, M.T., & Tang, D. (2013). DAMPs and autophagy: cellular adaptation to injury and unscheduled cell death. Autophagy, 9(4), 451-458.

Zwueste, D.M., Vernau, K.M., Vernau, W., Pypendop, B.H., Knych, H.K., Rodrigues, C.A., & Dickinson, P.J. (2023). Oral cytarabine ocfosfate pharmacokinetics and assessment of leukocyte biomarkers in normal dogs. Journal of Veterinary Internal Medicine, 37(6), 2429-2442.

APPENDIXES

APPENDIXES

Appendix (I): Serum Aspartate Aminotransferase determination

The activity of Aspartate aminotransferase (AST) was defined using AST Kit (BioSystems, Spain) (Schumann *et al.,* 2010).

Aspartate + 2 – Oxoglutarate ▸ Oxalacetate + Glutamate

Oxalacetate + NADH + H^+ ▸ (MDH) Malate + NAD^+

Procedure

Table of work solution of AST kit

Reaction temperature	37ºC	30°C
Working Reagent	1.0 mL	1.0 mL
Serum	50 µl	100 µl

1. The substrate B is added to substrate A and is mixed mildly to prepare the work solution.
2. The work solution is maintained at 30 °C.
3. The work solution and the serum are added into the tube, then mixed, and after one min, the first absorbance at min intervals is measured, and the next OD is recorded for three min.
4. The variation between successive absorbance and the mean absorbance difference per minute (ΔA/min) is determined.

Calculation

The level of the AST (GOT) in the serum is determined according to the following formula:

$$\Delta A/min \times \frac{Vt \times 106}{\varepsilon \times l \times VS} = U/L$$

The molar absorbance (ε) of NADH at 340 nm is 6300.

Light path (l) is 1 cm, and the total reaction volume (Vt) is 1.05 at 37°C and 1.1 at 30°C. Sample volume (Vs) is 0.05 at 37°C and 0.1 at 30°C, and 1 U/L is 0.0166 µkat/L.

The total reaction volume in light path

	37°C	30°C
ΔA/min	×3333= U/L ×55.55= µkat/L	×1746 = U/L ×29.1= µkat/L

Appendix (II): Serum Alanine Aminotransferase Activity (ALT) Determination:

Serum Alanine Aminotransferase activity ALT is determined by using a special kit (SPECTRUM ALT – kit, Egypt- IFUFCC25), by using device (Spectrophotometer Sesil, England).

Principle

Colorimetric determination of ALT activity is obtained according to the following reactions:

The pyruvate formed is measured in its derivative form, 2,4- dinitrophenylhydrazone

Reagents

Table: Components of ALT reagents

Reagent 1 ALT	Phosphate buffer pH 7.5 D-Alanine 2 Oxoglutarate Sodium Azide	100 mmol/l l200 mmol/l 6 mmol /l 12 mmol/l
Reagent 2 Color reagent	2.4 dinitrophe nylhydrazine	2 mmol /l

Procedure

Volume of ALT reagent 1, reagent 2 and sodium hydroxide. Wave length: 546 nm (530 – 550 nm)

Table of volume of ALT reagent 1

Reagent	Reagent blank	Sample
Reagent (Buffer)	0.5 ml	0.5 ml
Sample	**-------**	100 µl
Distilled water	100 µl	**------**

Mix and incubate for exactly 30 minutes at 37ºC.

Table of volume of ALT reagent 2

Reagent 2	0.5 ml	0.5 ml

Mix and incubate for exactly 20 minutes at 20-25ºC.

Table of volume of sodium hydroxide

Sodium hydroxide	5 ml	5 ml

Mix and measure absorbance of specimen against reagent blank at 546nm after 5 minutes.

Calculation

The ALT activity is obtaining from the following table:

Table of ALT absorbance

Absorbance	Value of ALT U/L	Absorbance	Value of ALT U/L
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.375	67
0.150	25	0.400	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

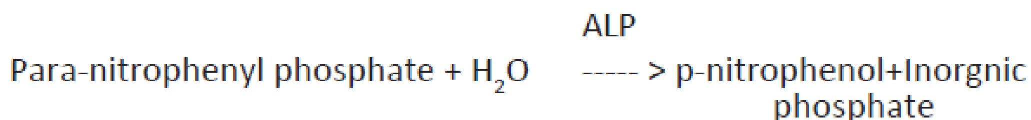
Linearity:

The assay is linear up to 94 U/L if the absorbance exceeds 0.5 at 546 nm, sample should be diluted 1+9 using sodium chloride and repeat the assay (result x 10).

Appendix (III): Serum Alkaline Phosphatase Activity Determination.

Principle

Kinetic determination of ALP according to the following reaction.



REAGENT COMPOSITION:

STORAGE AND STABILITY

The sealed reagents are stable upto the expiry date stated on the label,

When stored at 2-80C.

LINEARITY

The reagent is linear, upto 700 U/L.

If the concentration is greater than linearity (700 U/L), dilute the sample with normal saline & repeat the assay. Multiply the result with dilution factor.

NORMAL RANGE

It is recommended that each laboratory establish its own reference values. The following value may be used as guide line.

Male : 80 - 306 U/L

PREPARATION AND STABILITY OF WORKING REAGENT

Mix 4 volume of Reagent 1 (R1) with 1 volume Reagent 2 (R2) The working reagent is stable for 30 days at 2-80C.

Note: Discard the working reagent if the blank absorbance exceeds 1.00 at 405 nm.

PRECAUTION

To avoid contamination, use clean laboratory wares. Avoid direct exposure of working reagent to light.

Sample

Serum (free of haemolysis).

Antioxidant Induces Assessment

Appendix IV: Determination of Serum Malondialdehyde activity

Determination of Serum Malondialdehyde Level (MDA) Concentration (μ mol/L) Malondialdehyhe was estimated by Thiobarbituric acid (TBA) assay method (Muslih *et al.,* 2002) on spectrophotometer.

Principle

This method quantifies lipid peroxides by measuring aldehyde breakdown products of lipid peroxidation. Basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of thiobarbituric acid to form a red MDA-TBA complex which can be measure at 535 nm.

Stock TCA – TBA – HCl Reagent

It was prepared by dissolving 15% W/V trichloroacetic acid and 0.375% W/V thiobarbituric acid and 0.25 N HCl to make 100 ml (2.1 ml of concentrated HCl in 100 ml). This solution was mildly heated to assist in the dissolution of TBA.

Dissolved 15 gm TCA and 0.375 mg thiobarbituric acid in 0.25 N HCl and volume was made up to 100 ml with 0.25 N HCl.

Procedure

To 0.4 ml of serum, 0.6 ml TCA-TBA-HCl reagents were added. It was mixed well and kept in boiling water bath for 10 minutes. After cooling 1.0 ml freshly prepared 1N NaOH solution was added to eliminate centrifugation. This absorbance of pink color was measured at 535 nm against blank which contained distilled water in place of serum. In blank 0.4 ml distilled water and 0.6 ml TCA-TBA-HCl reagent was mixed and boiled. Blank was always taken.

Calculation

extinction coefficient of MDA at 535 nm is = 1.56×105

MDA concentration = χ / 0.0624 nmol / ml.

Appendix V: Determination of Serum Catalase activity

The concentration of the serum Catalase is defined based on the spectrophotometric according to Hadwan and Abed' s method (2018).

Reagents

Sulfuric acid solution (0.5 M) was prepared by appropriate dilution of concentrated sulfuric acid in 200 ml of distilled water.

Ammonium metavanadate solution (0.01 M) contained 0.2925 g of ammonium metavanadate in 200 ml of 0.5-M sulfuric acid.

Phosphate buffer (50 mM; pH 7.0) was prepared by mixing solutions a and b at a ratio of 1:1.5. Solution (a) was prepared by dissolving of 6.81 g of KH_2PO_4 in one liter of distilled water, and solution (b) was prepared by dissolving a 8.90 g of $Na_2HPO_4.2H_2O$ in one liter of distilled water.

Fresh H_2O_2 (10 mM) solutions were prepared by mixing 0.1134 ml of 30% H_2O_2 with 100 ml of phosphate buffer, and the solution was adjusted to 10-mM using the molar extinction coefficient of H_2O_2 at 240 nm (43.6 $M^{-1}cm^{-1}$).

Procedure

Table of the procedure for assessments of catalase activity

Reagents	Test	Standard	Blank
Sample	100 µl	------	-----
Phosphate buffer	900 µl	1000 µl	3000 µl
Hydrogen peroxide	2000 µl	2000 µl	----
Mix with vortex and incubate at 37 °C for 2 min, after that, add:			
Vanadium reagent	2000 µl	2000 µl	2000 µl
After that, the tubes were kept at 25 °C for 10 min. the changes in absorbance were recorded at 452 nm against the reagent blank.			

Calculation

Enzyme activity procedure was elucidated in (Table 1). The rate constant (k) of the first-order reaction equation for catalase activity was calculated using the following formula:

$$\text{Catalase Activity of test kU} = \frac{2.303}{t} * \log \frac{S^o}{S} \quad --- (1)$$

where t is time, S^0 is the absorbance of the standard solution, and S is the absorbance of the sample.

Appendix (VI): Determination of Serum Superoxide Dismutase activity

The activity of the Superoxide dismutase (SOD) enzyme was defined according to Marklund and Marklund's method (1974).

Preparation

1. Tris (0.258 gm) and Ethylene diamine tetra acetic acid (EDTA) (0.111gm) are dissolved into dH2O until yielding 100 ml Tris buffer (pH).

2. Pyrogallol (0.0252 g) was dissolved in HCL (10 mL), made up to 100 mL with dH2O, to obtain 100 mL of pyragallol (0.2 mM) solution.

Procedure

According to previous researchers (Assady *et al.*, 2011), to carry out the reaction, enzyme extract (50 µl), Tris buffer (2 ml), and pyrogallol (500 µl 0.2 mM) are required, and this mixture can absorb light at 420 nm. All items are present in the control sample, but the enzyme replaced deionized water, and the blank sample was considered distilled

water. An amount of enzyme that can inhibit 50% of pyrogallol oxidation is considered an enzyme unit.

To determine the activity of SOD, the equation is required:

SOD activity (u/ml) = (Vp-Vs)/(Vp*0.5) *(Vt/Vs) *n

The vp= Auto oxidation rate of pyrogallol (control)

Vs= Auto oxidation rate of the sample (with enzyme)

Vt=Total reaction volume (ml)

Vs= The volume of the required enzyme

n= dilution fold of the SOD sample

0.5= factor for 50% inhibition

Sex hormones Assay

Appendix (VII): Estimation of testosterone hormone concentration (ng/ml)

Principle:

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to DHT. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)- conjugated antibody specific for DHT is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain DHT and HRP conjugated DHT antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is

proportional to the concentration of DHT. You can calculate the concentration of DHT in the samples by comparing the OD of the samples to the standard curve.

Sample preparation

Serum preparation.

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2000 - 3000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

The test procedure was conducted in accordance with the following steps:

1. Dilution of Standards Ten wells are set for standards in a Microelisa stripplate. In Well 1 and Well 2, 100µl Standard solution and 50µl Standard Dilution buffer are added and mixed well. In Well 3 and Well 4, 100µl solution from Well 1 and Well 2 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. 50µl solution is discarded from Well 3 and Well 4. In Well 5 and Well 6, 50µl solution from Well 3 and Well 4 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. In Well 7 and ell 8, 50µl solution from Well 5 and Well 6 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. In Well 9 and Well 10, 50µl solution from Well 8 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. 50µl solution is discarded from Well 9 and Well 10. After dilution, the total volume in all the wells are 50µl and the concentrations are 480 pg/ml, 320 pg/ml,160 pg/ml, 80 pg/ml and 40 pg/ml, respectively

2. In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40μl Sample dilution buffer and 10μl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Incubation: incubate 30 min at 37℃ after sealed with Closure plate membrane.
4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Add 50 μl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3.
8. ashing as described in Step 5.
9. Coloring: Add 50 μl Chromogen Solution A and 50 μl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes. Please avoid light during coloring.
10. Termination: add 50 μl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Rat DHT were tested 20 times on one plate, respectively. Inter-assay

Precision (Precision between assays): 3 samples with low, middle and high level Rat DHT were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

Intra-Assay: CV<10%

Inter-Assay: CV<12%.

Appendix (VIII): Estimation of Follicle Stimulating Hormone Concentration (mIU/ml)

Principle.

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to FSH Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for FSH is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain FSH and HRP conjugated FSH antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of FSH You can calculate the concentration of FSH in the samples by comparing the OD of the samples to the standard curve.

Sample preparation

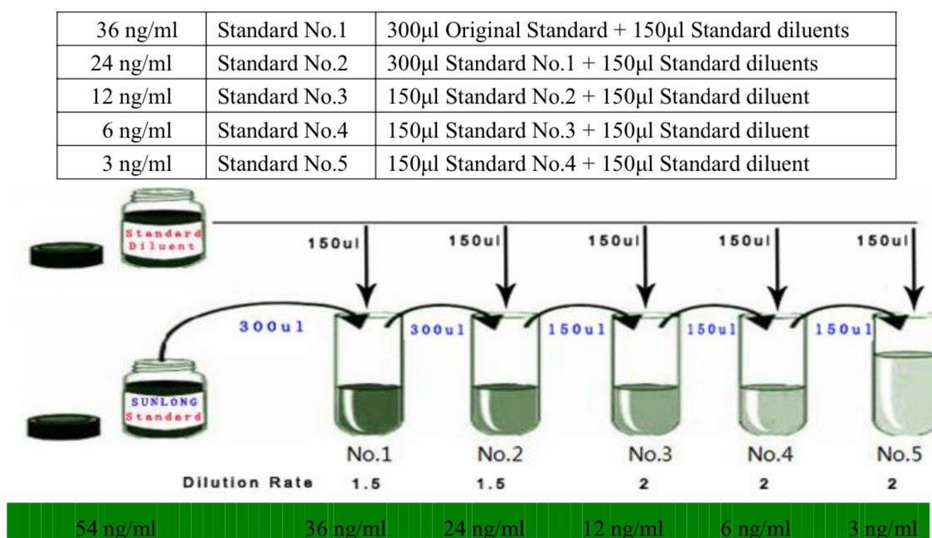
Serum preparation.

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2000 – 3000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

Procedure

1. Dilution of Standards

   Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well,each tube use two wells, total ten wells.



36 ng/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
24 ng/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
12 ng/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
6 ng/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
3 ng/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent

2. In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.

3. Incubation: incubate 30 min at 37℃ after sealed with Closure plate membrane.
4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and20 times for 48T).
5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Add 50 µl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37 ℃ for 15 minutes. Please avoid light during coloring.
10. Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes afteradding stop solution.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Rat DHT were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Rat DHT were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

Intra-Assay: CV<10%

Inter-Assay: CV<12%.

Appendix (IX): Estimation of Luteinizing Hormone Concentration (mIU/ml)

Principle.

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antigen specific to LH-Ab. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antigen. Then a Horseradish Peroxidase (HRP)-conjugated antigen specific for LH-Ab is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain LH-Ab and HRP conjugated LH antigen will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of LH-Ab. You can calculate the concentration of LH-Ab in the samples by comparing the OD of the samples to the standard curve.

Sample preparation

Serum preparation.

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2000 - 3000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

Procedure

1. Dilution of Standards

   Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube

   uses two wells, total ten wells.
2. In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40μl Sample dilution buffer and 10μl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Incubation: incubate 30 min at 37℃ after sealed with Closure plate membrane.
4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and
   20 times for 48T).
5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash
   solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Add 50 μl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Add 50 μl Chromogen Solution A and 50 μl Chromogen Solution B to each well, mix with gently shaking and incubate at 37 ℃ for 15 minutes. Please avoid light during coloring.

10. Termination: add 50 μl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. **Read** absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Rat DHT were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Rat DHT were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

Intra-Assay: CV<10%

Inter-Assay: CV<12%.

Appendix (X): Histological Study

Histological Technique (E&H) Stain

The testis and epididymis of each animal were quickly removed and rapidly weighed then prepared for histological study according to Mescher method, (2010) with aid of the light microscope as the following steps:

* **Fixation** The specimen fixated in the formalin 10% for 24–48 hours. In front of the bone marrow sample placed in a Bouin ‘s solution.

* **Washing and Dehydration** After fixation the specimens washed with water to remove the fixative in order to avoid the interaction between the fixative and staining materials used later. By dehydration the water had been completely extracted from fragments by bathing them successively in a graded series of ethanol and water (70%, 80%, 90%, and 100% ethanol).

* **Clearing** Bathing the dehydrated fragments in solvent (Xylene) for 30–60 minutes, this step was repeated 3 times. As the tissues clearing, they generally became transparent.

* **Infiltration and Embedding:** Once the tissue fragments were impregnated with the solvent, they were placed in melted paraffin in an oven, typically at 52 C. The heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin

* **Sectioning:** After holds from the oven, the specimen let at room temperature to be solid and removed from their containers in order to sectioning they were put in the rotary microtome and were sliced by the microtome, a steel blade into sections 5

micrometers thick. The sections were floated on water bath (50–55 oC), then transferred into glass slides coated with Mayers albumin as adhesive substance and left to dry.

* **Staining:** The histological sections of the studied organs were stained with HematoxylinEosin stain. Staining Procedure

1. Deparaffinize sections, 2 changes of xylene, 10 minutes each.

2. Re-hydrate in 2 changes of absolute alcohol, 5 minutes each.

3. 95% alcohol for 2 minutes and 70% alcohol for 2 minutes. 4. Wash briefly in distilled water.

5. Stain in Harris hematoxylin solution for 8 minutes.

6. Wash in running tap water for 5 minutes.

7. Differentiate in 1% acid alcohol for 30 seconds. 8. Wash running tap water for 1 minute.

9. Bluing in 0.2% Ammonia water or saturated Lithium Carbonate solution for 30 seconds to 1 minute.

10. Wash in running tap water for 5 minutes.

11. Rinse in 95% alcohol, 10 dips.

12. Counterstain in Eosin-Phloxine solution for 30 seconds to 1 minute.

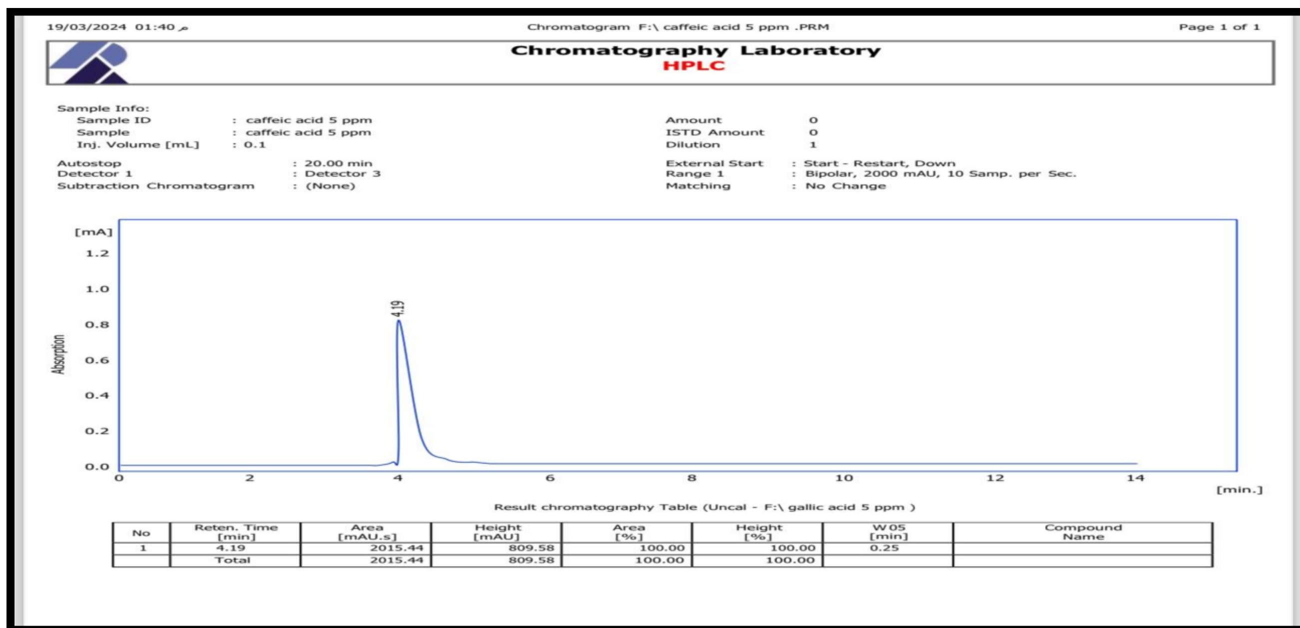
13. Dehydrate through 95% alcohol, 2 changes of absolute alcohol, 5 minutes each.

14. Clear in 2 changes of Xylene, 5 minutes each.

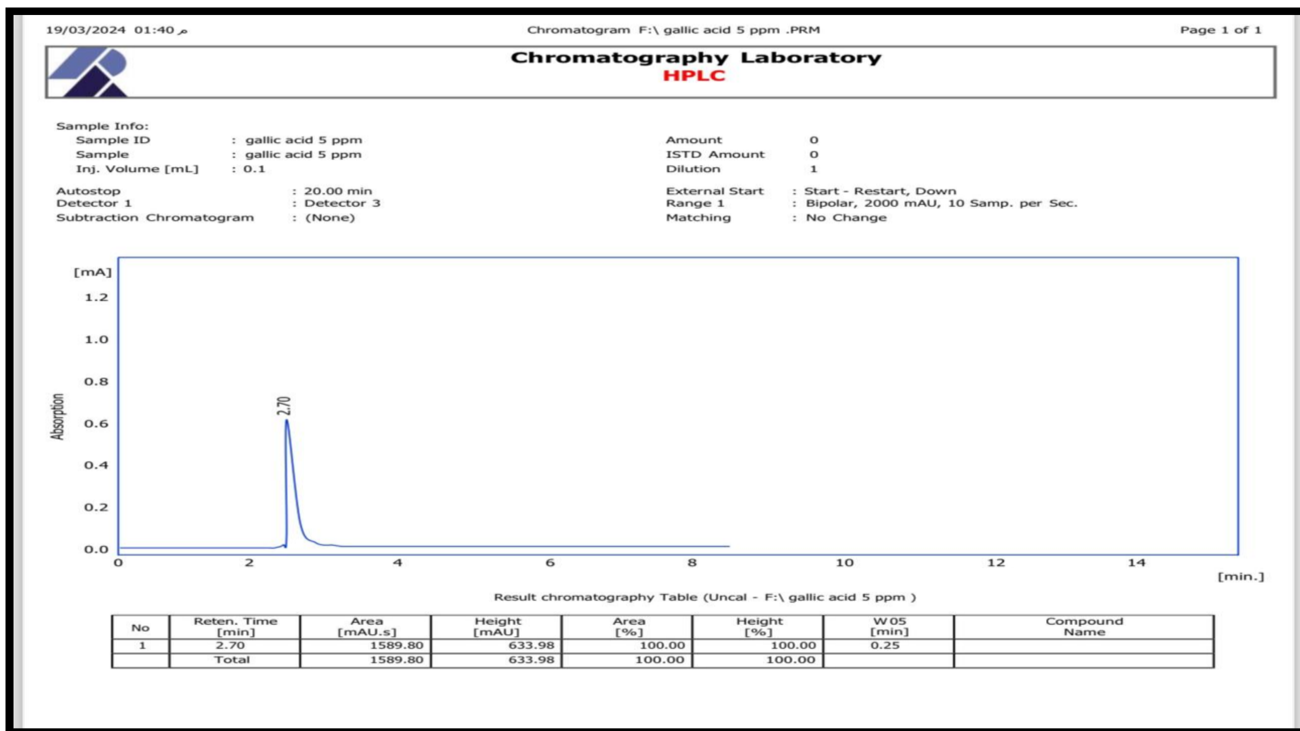
15. Mount with Xylene based mounting medium Trichrome stain (Modified masson's) procedure.

Quantification of individual phenolic compounds using HPLCanalysis of *T. terrestris*

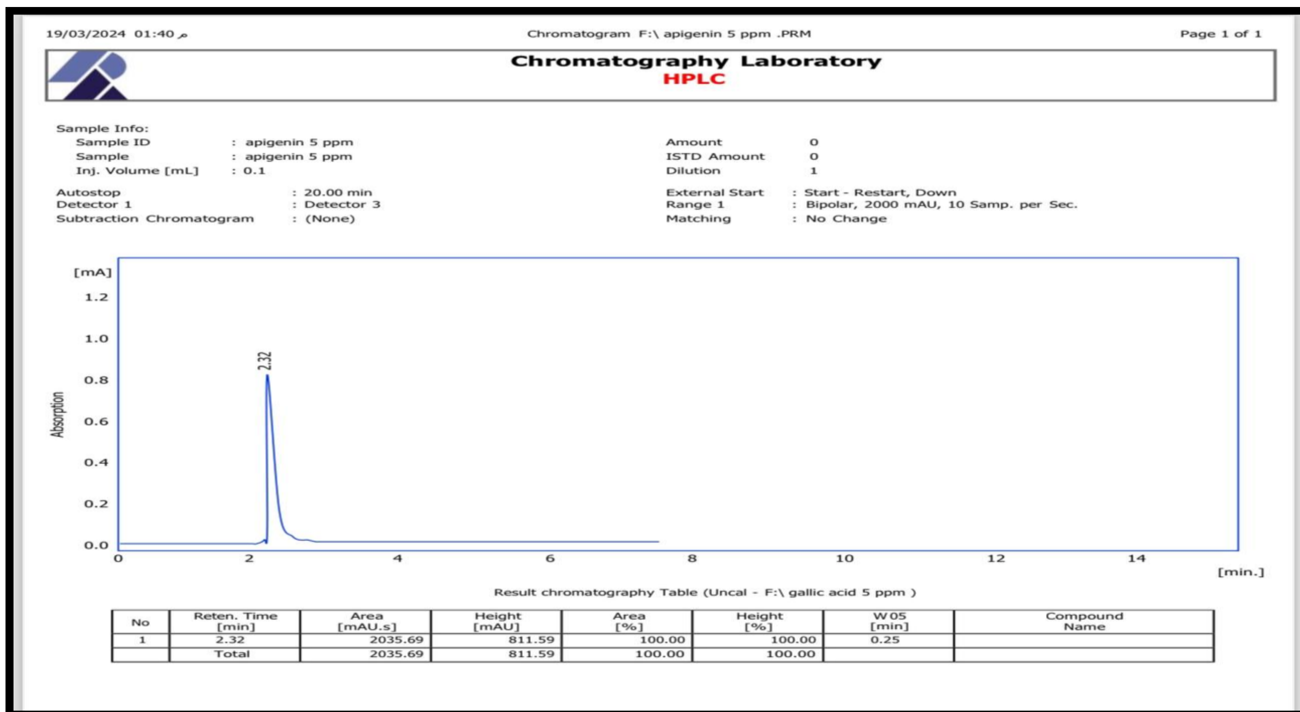
Quantification of individual phenolic compounds was performed by reversed phase HPLC analysis, using a SYKAM HPLC chromatographic system equipped with a UV detector), the column was -C18-OSD (25cm, 4.6mm). The column temperature was 30ºC the gradient elution method, with eluent A (methanol) and eluent B (1% formic acid in water (v/v)) was performed, as follows: initial 0-4 min, 40 % B ; 4-10 min, 50 % B; . and flow-rate of 1.0 mL/min. The injected volume of samples 100 µL .and standards was 100 µL and it was done automatically using autosampler. The spectra were acquired in the 280 nm.



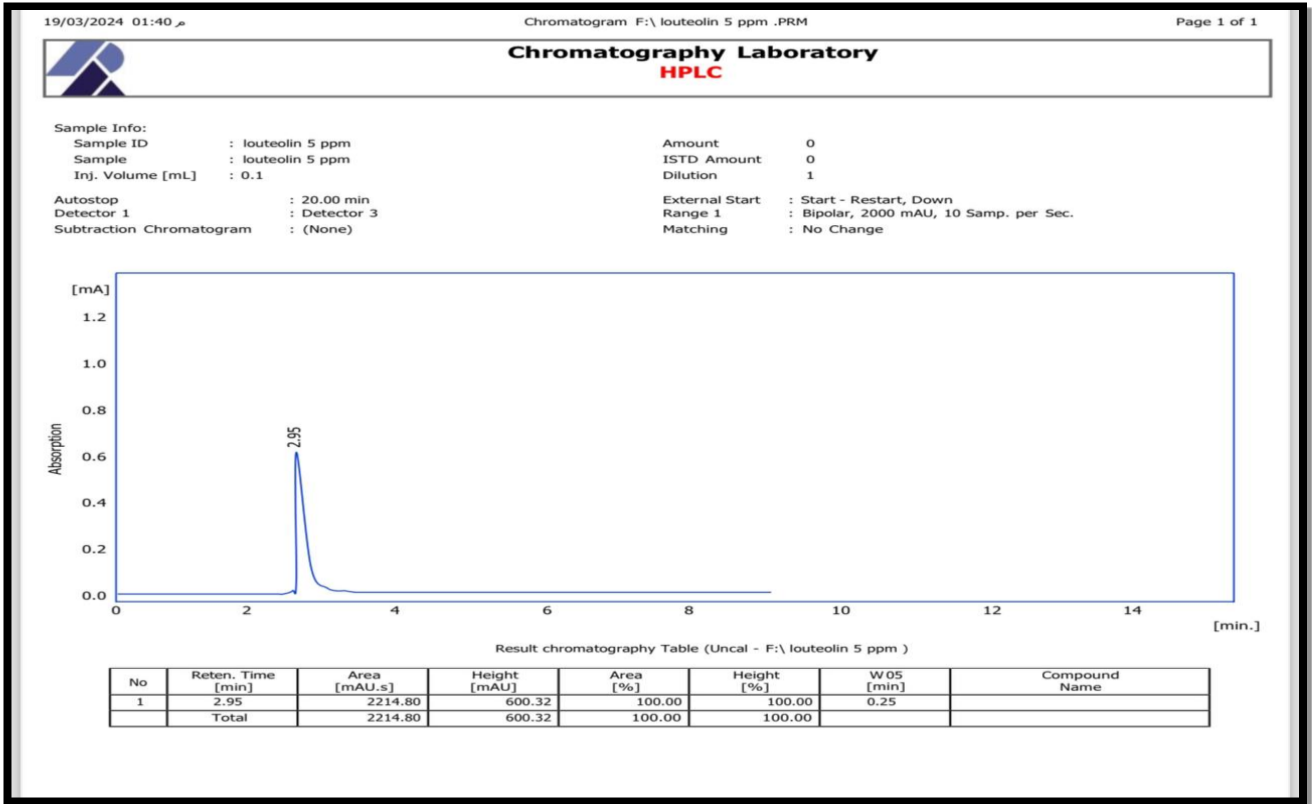
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.19	2015.44	809.58	100.00	100.00	0.25	
	Total	2015.44	809.58	100.00	100.00		



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.70	1589.80	633.98	100.00	100.00	0.25	
	Total	1589.80	633.98	100.00	100.00		



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	2.32	2035.69	811.59	100.00	100.00	0.25	
	Total	2035.69	811.59	100.00	100.00		



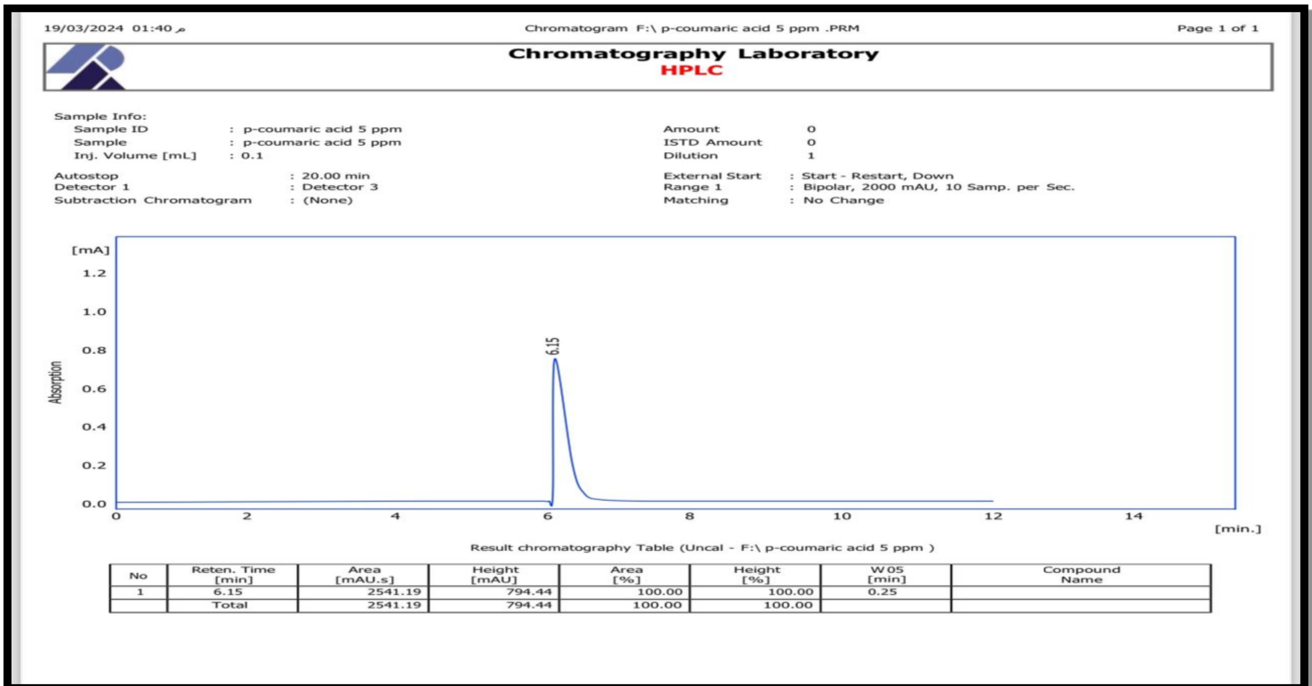
19/03/2024 01:40 م   Chromatogram F:\ louteolin 5 ppm .PRM   Page 1 of 1

Chromatography Laboratory
HPLC

Sample Info:
Sample ID : louteolin 5 ppm
Sample : louteolin 5 ppm
Inj. Volume [mL] : 0.1
Amount 0
ISTD Amount 0
Dilution 1
Autostop : 20.00 min
Detector 1 : Detector 3
Subtraction Chromatogram : (None)
External Start : Start - Restart, Down
Range 1 : Bipolar, 2000 mAU, 10 Samp. per Sec.
Matching : No Change

Result chromatography Table (Uncal - F:\ louteolin 5 ppm)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.95	2214.80	600.32	100.00	100.00	0.25	
	Total	2214.80	600.32	100.00	100.00		



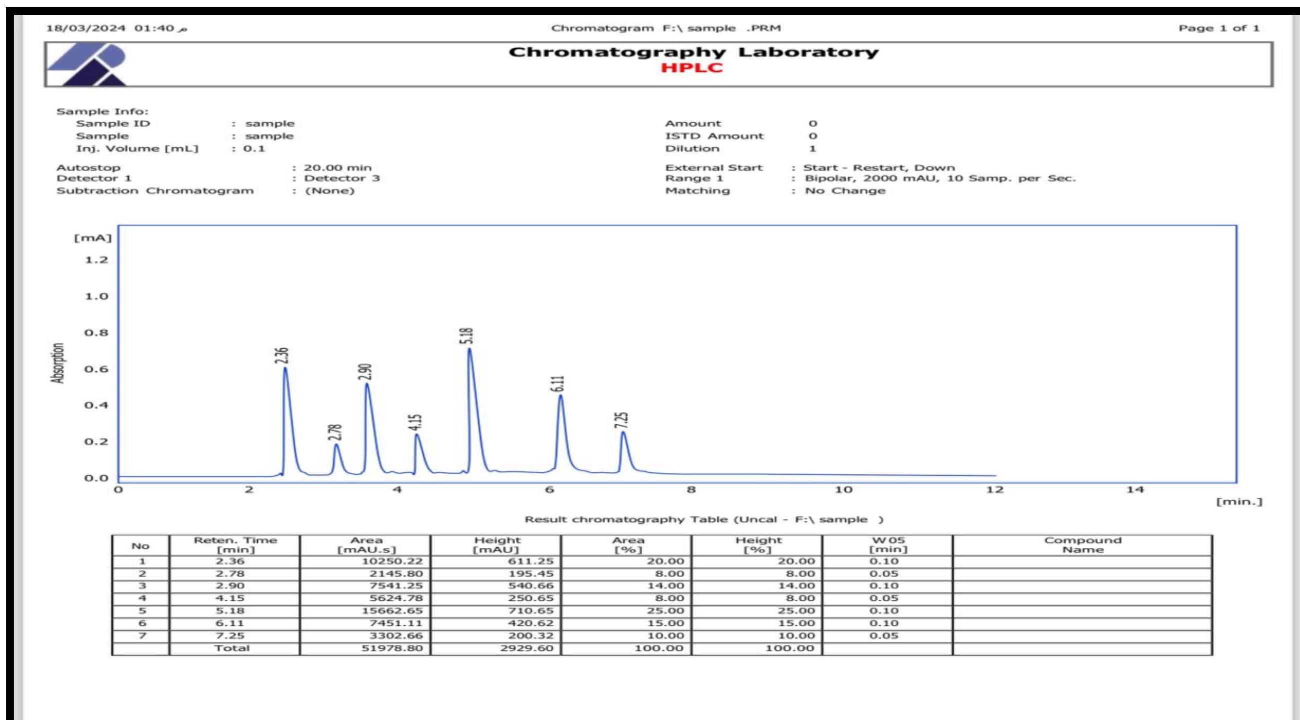
19/03/2024 01:40 م   Chromatogram F:\ p-coumaric acid 5 ppm .PRM   Page 1 of 1

Chromatography Laboratory
HPLC

Sample Info:
Sample ID : p-coumaric acid 5 ppm
Sample : p-coumaric acid 5 ppm
Inj. Volume [mL] : 0.1
Amount 0
ISTD Amount 0
Dilution 1
Autostop : 20.00 min
Detector 1 : Detector 3
Subtraction Chromatogram : (None)
External Start : Start - Restart, Down
Range 1 : Bipolar, 2000 mAU, 10 Samp. per Sec.
Matching : No Change

Result chromatography Table (Uncal - F:\ p-coumaric acid 5 ppm)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	6.15	2541.19	794.44	100.00	100.00	0.25	
	Total	2541.19	794.44	100.00	100.00		


18/03/2024 01:40 م   Chromatogram F:\ sample .PRM   Page 1 of 1

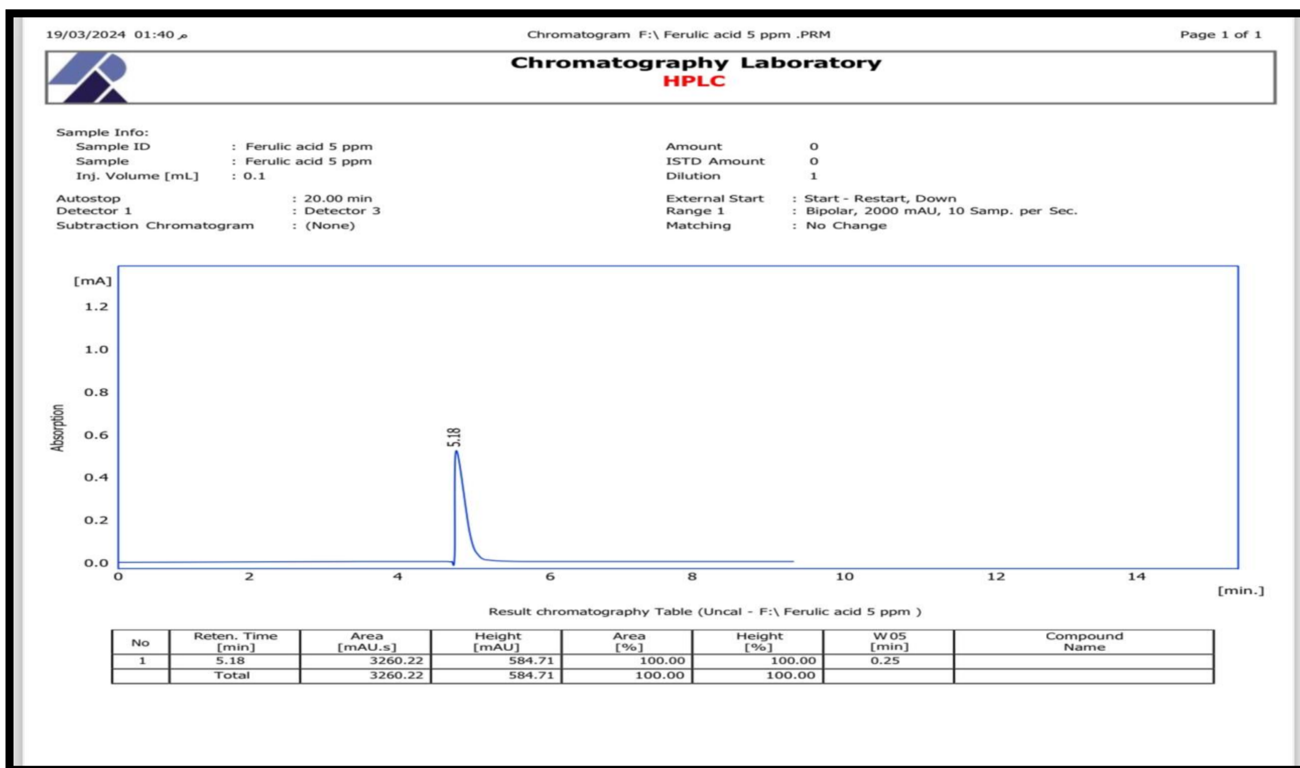
Chromatography Laboratory
HPLC

Sample Info:
Sample ID : sample
Sample : sample
Inj. Volume [mL] : 0.1
Amount 0
ISTD Amount 0
Dilution 1

Autostop : 20.00 min
Detector 1 : Detector 3
Subtraction Chromatogram : (None)
External Start : Start - Restart, Down
Range 1 : Bipolar, 2000 mAU, 10 Samp. per Sec.
Matching : No Change

Result chromatography Table (Uncal - F:\ sample)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.36	10250.22	611.25	20.00	20.00	0.10	
2	2.78	2145.80	195.45	8.00	8.00	0.05	
3	2.90	7541.25	540.66	14.00	14.00	0.10	
4	4.15	5624.78	250.65	8.00	8.00	0.05	
5	5.18	15662.65	710.65	25.00	25.00	0.10	
6	6.11	7451.11	420.62	15.00	15.00	0.10	
7	7.25	3302.66	200.32	10.00	10.00	0.05	
	Total	51978.80	2929.60	100.00	100.00		


19/03/2024 01:40 م   Chromatogram F:\ Ferulic acid 5 ppm .PRM   Page 1 of 1

Chromatography Laboratory
HPLC

Sample Info:
Sample ID : Ferulic acid 5 ppm
Sample : Ferulic acid 5 ppm
Inj. Volume [mL] : 0.1
Amount 0
ISTD Amount 0
Dilution 1

Autostop : 20.00 min
Detector 1 : Detector 3
Subtraction Chromatogram : (None)
External Start : Start - Restart, Down
Range 1 : Bipolar, 2000 mAU, 10 Samp. per Sec.
Matching : No Change

Result chromatography Table (Uncal - F:\ Ferulic acid 5 ppm)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.18	3260.22	584.71	100.00	100.00	0.25	
	Total	3260.22	584.71	100.00	100.00		

الخلاصـــــة

النماذج الحيوانية ذات قيمة كبيرة لتحقيق وتحديد الأهداف وتطوير أساليب صيدلانية جديدة. الدراسة الحالية في المزايا العلاجية المحتملة، والفعالية كمضاد للأكسدة، والتأثيرات الوقائية ضد الخلل الإنجابي المستحث كيميائيًا على تلف تكوين النطف. يمكن أن تؤدي التأثيرات السامة للخلايا للعديد من علاجات السرطان إلى عواقب سلبية عامة مختلفة وضعف في تكوين النطف. ولذلك، فإنه يظل مصدر قلق هام يحتاج إلى مزيد من التحسين. السيتارابين (Ara C) ، وهو دواء علاج كيميائي يستخدم على نطاق واسع للأورام الدموية الخبيثة، له آثار ضارة على التطور الإنجابي للذكور ووظيفته عن طريق كبت تكاثر الخلايا الجرثومية وتحفيز موت الخلايا المبرمج. سيتم تقسيم عشرين من الجرذان الذكور الناضجة إلى أربع مجموعات. تحتوي كل مجموعة على 5 جرذان. المجموعة الأولى هي مجموعة ضابطة، المجموعة الثانية جرعت بمستخلص *T. terrestris* 250 ملغم/كغم) والمجموعة الثالثة جرعت بـ 25 Ara-C ملغم/كغم من وزن الجسم) والمجموعة الرابعة جرعت Ara - C (25 ملغم/كغم من وزن الجسم) داخل الصفاق + مستخلص *T. terrestris* 250 ملغم/كغم من وزن الجسم) عن طريق الفم لمدة 28 يوماً. كشف فحص HPLC للمستخلص الجاف لـ TT عن وجود مجموعة متنوعة من المركبات الفينولية. يهدف عملنا إلى استكشاف وظيفة الحماية TTE ضد ضعف الإنجاب الناجم عن Ara C في ذكور الجرذان البالغة. تسبب Ara C في زيادة تعتمد على الجرعة في أنشطة إنزيمات الكبد في الدم AST) وALT و (ALP ؛ ارتفاع الإجهاد التأكسدي. إعاقة تكوين الستيرويد (انخفاض هرمون التستوستيرون، الهرمون المنبه للجريب، الهرمون الاصفري. علاوة على ذلك، أدى التعرض المباشر لـ Ara-C إلى انخفاض في نشاط 17 β-HSD، والذي يمكن ربطه بانخفاض مستويات هرمون التستوستيرون في الدم، وفقًا للتحليل النسيجي ظهرت تشوهات في الخصيتين، وجودة السائل المنوي، وارتفاع مستوى الكاسبيز مقارنة بالمجموعات الأخرى.

في الختام، كشف Ara-C عن انخفاض تكوين النطف الذي تم اكتشافه وسبب تلف الخصية. وعمل مستخلص عشبة تريبولوس تيريستريس TTE على تقليل الاعراض الجانبية للسيتارابين. أكد البحث الحالي على أهمية TTE في تحسين التغيرات في خصية الجرذان التي يسببها حقن Ara-C، ربما بسبب وجود مركبات مضادة للأكسدة.

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الطب البيطري

فرع الفسلجة والكيمياء الحياتية والأدوية

التأثير الوقائي للمستخلص الكحولي لعشبة تريبولوس تيريستريس ضد الخلل الوظيفي في الكبد والخصية المستحدث بواسطة السيترابين في ذكور الجرذان

رسالة مقدمة الى مجلس كلية الطب البيطري / جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في اختصاص علوم الطب البيطري/ الفسلجة

بواسطة

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أ. م. د

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