The concept of metabolic activation and metabolic alteration has been a very useful paradigm in chemical induced toxicity for several reasons - first, it has provided a mechanistic basis for understanding the initial action of structurally diverse chemicals and toxicants; second, it has provided an explanation for the binding of many chemical compounds to DNA and other macromolecules; and third, it has been a crucial bit of knowledge in the creation of short-term genotoxicity.

In the last few decades, indiscriminate use of some habit-forming and anabolic drugs has become much more widespread but has nevertheless remained covert and intermittent involving an increasing number of agents. Consequently, clinical information concerning the effects of these recreational drugs has continued to be derived from isolated case reports. These uncontrolled anecdotal observations make it difficult to draw unequivocal conclusions. The virtual absence of long-term perspective data also makes uncertain judgements. There may also be significant differences in the dose-response characteristics between individual drug-dependent actions and target tissue. Some individuals use extremely high doses of these drugs, producing plasma concentrations two or three orders of magnitude greater than that found in healthy men. At these non-physiological concentrations the receptors would already have been saturated, enzyme activity have been altered and any biological response may well have reached a plateau, while at the same time atypical actions can be induced. The endocrine homeostasis encountered in drug abuse is typically in orders of magnitude higher or lower than physiological concentrations. However, exact dose-response relations of individual drug actions are not fully characterized.

Even though different mechanisms may mediate the action of these drugs, the unwanted side-effects including effects on reproductive health cannot be completely dissociated
from their euphoric and anabolic effects. The adverse effects of illicit use of these drugs, however, depend on the age and sex of the individual, the duration and total dose of exposure, and the type of drug used. The unwanted consequences of drug misuse are most damaging in adolescents and are associated with many undesirable endocrinological effects. Suppression of the hypothalamic-pituitary-gonadal axis that in severity results in infertility may sometimes be irreversible.

The extensive and indiscriminate use of these chemical substances, often natural and sometimes designed by human intellect for prescription use, has boomeranged on the human society. The change in the neural co-ordination simultaneously with chemical coordination through endocrine system can easily take place not only in the course of prolonged adaptation, but also in the course of fast encounter with these hazards. The physiological status of organism running in unison with a concerted tune is ruined by affecting its component system.

Drug - a term of varied usage which, in medicine, refers to any substance with the potential to prevent or cure disease or enhance physical or mental welfare. In pharmacology, it refers to any chemical substance that alters the biochemical or physiological processes of tissues or organisms. The United Nations drug control conventions do not recognize a distinction between licit and illicit drugs. The term illicit drugs is used to describe drugs which are under international control, may or may not have licit medical purposes, but which are produced, trafficked and consumed illicitly. A number of drugs of widely varying chemical structures and physical and chemical properties are known to produce compulsive physical and psychological dependence as a result of which the user feels an urge to use more of the substance to preserve the original effect and when the dependent user stops taking the drug, a “withdrawal
reaction” sets in, implying a biochemical change in the body that can manifest physical and psychological symptoms. Such type of substances are referred to as habit-forming drugs which, due to their properties, cause their user to become dependent upon them by habit or addiction upon excess or repeat intakes. These substances include an array of chemicals ranging from ethanol, tobacco and caffeine to amphetamines and opiates. They include hallucinogens, narcotics, stimulants, sedatives and hypnotics.

People abuse habit forming substances such as alcohol and tobacco for varied and complicated reasons but it is clear that our society pays a significant cost. Abused substances produce some form of intoxication that alters judgement, perception, attention or physical control. Nearly all drugs of abuse directly or indirectly target the brain’s reward system by flooding the circuit with dopamine. This neurotransmitter produces the euphoric effects sought by people who abuse drugs and encourages them to repeat the behaviour, thus leading to addiction.

The abuse of anabolic drugs or anabolic androgenic steroids (AAS), the synthetic derivatives of testosterone, is under constant debate worldwide as a large number of young adolescents abuse AAS in order to improve their physical fitness and appearance. While formerly restricted to competing athletes, now AAS are also abused by non-competitive adolescent athletes as well as non-athletic sub-groups to gain euphoria (Terney and Mc Lain, 1990; Handelsman and Gupta, 1997; Korkia and Stimson, 1997; Kindlundh et al., 1999; Nilsson et al., 2001; Tan and Scally, 2009).

Although prevalence estimates vary, there is no doubt that the use of both licit and illicit substances is linked to a range of individual and community harms that impose significant costs. Drug abuse is well known to cause damage to the entire human body. People may not be aware of the havoc it can wreak on the reproductive system for both
males and females. Recreational use of illicit drugs is an important consideration when assessing the etiology of male infertility. In fact, it is believed that drug use plays a role in a large percentage of many unexplained fertility cases. Exposure to certain drugs like anabolic steroids, alcohol and tobacco can have a very negative impact on both male and female reproductive system (Torres-Calleja et al., 2001; Sadeu et al., 2010).

Infertility, a critical component of reproductive health, is a major public health problem in the modern society which affects 10-15% of all couples across the globe (Mosher and Pratt, 1991). Male factor infertility is considered to be the sole contributing factor in conception difficulties of up to 40% of infertile couples (Yesilli et al., 2005). Male fertility depends on the proper functioning of a complex system of organs and local balance between androgen and estrogen which is very essential for spermatogenesis. Male reproductive pathologies can be congenital or acquired, characterized by reduced sperm count due to impaired spermatogenesis or abnormal maturation, and sperm dysfunction caused by metabolic deregulation or oxidative stress (Rowe, 2006; Lekamge et al., 2008; Desai et al., 2009; Saalu, 2010). Molecular mechanism of male factor infertility suggests that high levels of reactive oxygen species (ROS) generated by various sources leads to infertility by initiating apoptosis of mature germ cells and also by damaging the sperm genetic material (Sun et al., 1997; Agarwal and Saleh, 2002; Sharma et al., 2004).

Although in some men a specific disorder may be present, in 30-40% of them no apparent reason for infertility has been reported. In the present-day scenario, lifestyle factors like illicit drug use is considered to be an important cause of male factor infertility which includes the use of anabolic-androgenic steroids (AAS), alcohol, nicotine, marijuana, opioid narcotics, cocaine etc. (Fronczak et al., 2012).
Anabolic-androgenic steroids (AAS) are pharmacologically important for their use in the treatment of various medical conditions such as growth deficiency, some blood disorders, osteoporosis, breast cancer, growth promotion, hypogonadal dysfunction and the commencement of delayed puberty in men (Clark et al., 1997; Feinberg et al., 1997; Thiblin and Petersson, 2005; Tan and Scally, 2009). However, AAS are not always used purely for medical purposes, these are the most commonly abused drug used by some athletes, of which Nandrolone Decanoate is one of the most common (Lippi and Guidi, 1999; Tan and Scally, 2009). The metabolism of nandrolone was investigated by Engel et al. (1958). The metabolism strongly follows that of testosterone, and the main metabolites are found to be 3α-hydroxy-5α-estran-17-one (norandrosterone) and 3α-hydroxy-5β-estran-17-one (noretiocholanolone), the structures of which were elucidated by Kupfer et al. (1960). Besides these metabolites, a 3β-hydroxy isomer, 3β-hydroxy-5α-estran-17-one, is also excreted into urine as a 3β-sulfate in an amount similar to that of the 3α-hydroxy metabolites (Fig. I.2)

![Fig. I.1: Common Anabolic steroids.](image-url)
AAS are taken by abusing athletes at supraphysiological doses which are usually 10 to 100 fold the recommended therapeutic dose (Clark et al., 1997). Traditionally, AAS have been abused in drug-use cycles of 6-14 weeks followed by a drug-free period to prevent building up tolerance to AAS (Karila et al., 2004).

The exact adverse effects of AAS abuse is very hard to define due to lack of clinical trials which mimic AAS abuse by athletes (Hall and Hall, 2005). Almost all major tissues in the body including the brain, have androgen receptors, thus AAS abuse affects almost all body systems (Karila et al., 2004). Due to widespread abuse, many side-effects of AAS may turn out to be significant risk factors when considering public health (Yesalis et al., 1989). Many AAS-induced adverse effects are considered to be reversible.

Reports indicate that AAS abuse results in a wide range of physiological and psychological effects such as altered behaviour in terms of increased aggression,
irritability, cognitive dysfunction, hypomania and addictive behaviour (Bahrke et al., 1990; Pope et al., 2000). Adverse physiological effects like baldness and dermatological effects such as acne and striae are very common among users administering very high doses of AAS (Scott and Scott, 1992). Side effects of AAS abuse include liver failure, a decrease in high density lipoprotein (HDL) levels and hepatic adenomas, mood fluctuation, violence and suicide attempts (Clark et al., 1997; Boyadjiev et al., 2000; Hall and Hall, 2005). Moreover, there are numerous case reports of myocardial infarction, coronary atherosclerosis, congestive heart disease, serious arrhythmia, atrial fibrillation, intraventricular thrombosis, pulmonary embolus and arterial and venous thrombosis associated with AAS abuse (McNutt et al., 1988; Ferenchick, 1991; Ferenchick and Adelman, 1992; Gaede and Montine, 1992; Appleby et al., 1994; Huie, 1994; Mewis et al., 1996; Niimenen et al., 1996; Ferrera et al., 1997; Sullivan et al., 1999; Fineschi et al., 2001). AAS have also been reported to be aetiological factors for some cancers. Hepatocellular carcinoma is connected to long term treatment with AAS (Ishak and Zimmerman, 1987). There are contradictory evidences about the role of androgens in prostate cancer (Signorello et al., 1997; Heikkila, 1999). Reports also indicate association of AAS with development of soft tissue sarcomas (Zahm and Fraumeni, 1997). However, regression occurs in majority of cases after withdrawal of AAS administration (Shahidi, 2001).

AAS abuse impacts upon several hormonal systems, most notably the hypothalamic-pituitary-thyroid (HPT), hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes. Reports indicate marked decrease in thyroid binding globulin accompanying AAS administration with decrease in T₃ and T₄ (Clerico et al., 1981; Small et al., 1984; Alen et al., 1985, 1987; Deyssig and Weisell, 1993). Reported
effects of AAS administration on the HPA axis appear to be complex and conflicting. Some studies indicate increased cortisol levels, decrease in adrenal androgen DHEA (dehydroepiandrosterone) and ACTH (adreno-corticotropic hormone) whereas others indicate no change in cortisol, ACTH and DHEA levels (Hervey et al., 1976; Alen et al., 1985; Ruokonen et al., 1985, Daly et al., 2003). One of the most pronounced effects of AAS abuse is its negative impact on the hypothalamic-pituitary-gonadal axis (Takahashi et al., 2004). AAS via negative feedback to hypothalamus induce hypogonadotrophic-hypogonadism associated with decreased serum testosterone concentrations, testicular atrophy, impaired steroidogenesis and spermatogenesis (Kilshaw et al., 1975; Schurmeyer et al., 1984; Jarow and Lipshultz, 1990). On AAS administration, there is marked depression of serum testosterone, sex hormone-binding globulin (SHBG) as well as gonadotrophins i.e., luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which control steroidogenesis and spermatogenesis (de Kretser et al., 1998, 2000; Sader et al., 2001). Reports indicate that normal spermatogenesis requires a 50-fold higher androgen concentration in the testes than in the peripheral serum (Adamopoulos et al., 1984; Turner et al., 1984).

AAS abuse induces oligozoospermia and azoospermia. It has been reported that AAS abuse not only reduces sperm concentration, but also impairs the percentage of morphologically normal semen. AAS-induced hypogonadotrophic hypogonadism also results in lower semen density (Schurmeyer et al., 1984; Torres-Calleja et al., 2001). AAS administration also induce changes in other hormone levels and endocrinological systems, probably mediated by multiple receptor interactions, but these effects seem to be reversible (O’Connor et al., 1990; Brower, 1993).
The stringent legislative regulations imposed by health regulatory authorities over the production and use of opiates, cannabinoids, and their synthetic analogues have resulted into an increased consumption of substances like tobacco and alcohol. Tobacco (*Nicotiana tabacum*) and alcohol (ethanol) are considered to be the largest consumed addictive substances by the people irrespective of age, sex, social and economic status, in all sub-continents of the globe. Reports indicate that people having stress, anxiety and depression are more vulnerable to seek refuge in tobacco and alcohol (Miller and Gold, 1998). The principal source of nicotine exposure is through the use of tobacco (Heisheman *et al*., 1994). Nicotine is the main alkaloid constituent of tobacco that is based on the five member pyrrolidine and six member pyridine structures. It is a subgroup of pyridine and piperidine alkaloids which is derived from vitamin B$_3$ (Nicotinic acid) (Yildiz, 2004).

**Fig. I.3: Nicotine.**

Nicotine is absorbed through the respiratory tract, mouth mucosa and skin. Approximately, 80 – 90 % of nicotine gets metabolized by body organs, mainly by the liver, and also by the kidney and lungs (Kyerematen *et al*., 1990; Benowitz *et al*., 2006). Nicotine is metabolized to cotinine in vivo within minutes after its absorption, with cotinine having a half-life of 19-24 hours in rodents (Sastry *et al*., 1995). Nicotine and its metabolites have been detected in serum, urine, saliva, milk and also in smoker’s seminal plasma (Pacifici *et al*., 1995).
Cigarette smoke is a complex mixture of toxic chemicals including nicotine, cadmium, carbon monoxide, ammonia, volatile hydrocarbons, aldehyde and several recognized carcinogens and mutagens (Stedman, 1968). These harmful components are absorbed through the pulmonary vasculature and transported via blood stream causing cytotoxicity, genotoxicity and tumorigenicity throughout the body (Stillman et al., 1986; Clair et al., 1994). Smokeless tobacco contains several carcinogens, of which tobacco-specific N-nitrosamine (TSNA), N’-nitrosonornicotine (NNN) and 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK) are the most important (Hoffmann and Djordjevic, 1997).
Studies confirm that upon chronic administration, nicotine produces dependence leading to addiction (Henningfield et al., 1995). Nicotine activates the mesolimbic dopamine system resulting in dopamine release in the nucleus accumbens by stimulating nicotine acetylcholine receptors on the cell bodies of mesolimbic dopamine neurons leading to increased extracellular dopamine levels which mediate the abuse related effects (Corrigall et al., 1992; Rose and Corrigall, 1997; Di Chiara, 2000; Watkins et al., 2000). It has been reported by several workers that nicotine affects a wide range of cellular processes from gene expression to secretion of hormones and modulation of enzymatic activity (Sano et al., 1999; Yildiz et al., 1999; Zhang et al., 2001). Chronic systemic exposure to nicotine also contributes to accelerated coronary artery disease, acute cardiac ischemic events and hypertension (Benowitz, 1997). Numerous studies have shown that in addition to the deleterious effects on cardiovascular and pulmonary physiology, excessive cigarette smoking affects the female reproductive system and imposes a number of risks specific to women viz., infertility, ectopic pregnancy, menstrual abnormalities and early onset of menopause (Midgette and Baron, 1990; Kendric et al., 1996; Saraiya et al., 1998).

A number of studies have shown that long term chronic intake of nicotine has serious detrimental effects on male sexual functioning. Chronic users suffer from impotence, loss of libido, premature or delayed ejaculation, decline in testosterone levels, decreased sperm count and sperm maturation, thereby, affecting sexual potential in the form of infertility. Chronic treatments with nicotine have been reported to cause decline in fertilizability in male animals (Patterson et al., 1990; Yamamoto et al., 1998). Studies indicate that cigarette smoking negatively affects every system involved in the male reproductive process. Spermatozoa from smokers have reduced fertilizing capacity.
Smokers demonstrate lower semen volume, sperm count, sperm motility, viability and a reduced percentage of morphologically normal sperm compared with non-smokers. Smokers are reported to show increased seminal leucocytes, oval sperm percentage, spermatozoa with defective head piece and with cytoplasmic droplets (Close et al., 1990; Holzki et al., 1991; Lewin et al., 1991; Moskova and Popov, 1993; Sofikitis et al., 1995; Ochedalski et al., 1994; Vine et al., 1996; Merino et al., 1998; Mak et al., 2000; Zinaman et al., 2000; Trummer et al., 2002; Kunzle et al., 2003; Hassa et al., 2006; Bouvet et al., 2007; Colagar et al., 2007; Gaur et al., 2007; Hansen et al., 2007; Hassan et al., 2009).

Smokers have seminal cotinine and trans-3’-hydroxycotinine levels similar to the serum, while there is significant increase in seminal nicotine as compared to the serum. Total sperm motility is reported to be negatively correlated to seminal cotinine and trans-3’-hydroxycotinine levels (Pacifici et al., 1993). Ascorbic acid is considered to be the most essential antioxidant in semen, with human seminal plasma containing 10 mg/dl ascorbic acid which is 9 times its concentration in blood plasma. Heavy smoking leads to 20 – 40% decrease in serum ascorbic acid, and ascorbic acid supplement leads to improved sperm quality (Smith and Hodges, 1987; Dawson et al., 1992). Seminal zinc, copper and superoxide dismutase (SOD) have been reported to be much lower in medium, heavy and long term smokers than non-smokers, being negatively correlated with the amount and duration of cigarette smoking (Zhang et al., 2000). Smoking leads to 48% increase in seminal leucocytes and 107% increase in reactive oxygen species (ROS) in the semen. Leucocytes are the major source of reactive oxygen species (ROS) in the semen (Sharma and Agarwal, 1996). Elevated levels of leucocytes may impair fertility by formation of reactive oxygen species which are harmful to sperm DNA and
membrane phospholipids because of oxidation (Kim and Parthasarathy, 1998; Ochsendorf, 1999; Shen et al., 1999). Sepaniak et al. (2004) reported that the active transfer of cigarette components through the blood-testis barrier possibly induce oxidative stress-induced DNA damage which is one of the causes of sperm quality alteration.

Reports indicate that the combination of smoking and varicocele is strongly related to the incidence of oligozoospermia. Male smokers with varicocele are reported to have an incidence of oligozoospermia 10 times greater than non-smokers and five times greater than men who smoke but are free of varicocele (Klaiber et al., 1987). Moreover, nicotine alters the hypothalamic-pituitary axis through stimulation of growth hormone, cortisol, and vasopressin and oxytocin release, which in turn inhibit LH and PRL release (Weisberg, 1985). The mean 17 beta-estradiol (E) level is reported to be higher and the mean levels of LH, FSH and PRL are lower in smokers compared with non-smokers (Ochedalski et al., 1994). Trummer et al. (2002) reported increased free and total serum levels of testosterone and decreased PRL in smokers.

On the other hand, a handful of studies have found no association between nicotine and sperm quality or sperm function as well as serum testosterone levels (Vogt et al., 1986; Dikshit et al. 1987; Lewin et al., 1991). Available data do not conclusively demonstrate that smoking decreases male fertility. However, it is regarded as an infertility risk factor (Vine, 1996).

Since ancient times alcoholic beverages have been closely associated with diet, particularly in Mediterranean countries, and for many years, moderate and regular consumption of alcohol has been associated with health benefits, with no scientific basis (Willett et al., 1995). However, over the last two decades, numerous studies have
demonstrated that intake of alcoholic beverages produces positive effects on antioxidant capacity, lipid profile and the coagulation system, resulting in reduction in the risk of cardiovascular disease (CVD), overall mortality and other diseases in moderate drinkers (Friedman and Kimball, 1986; Gronbaek et al., 1995; Muntwyler et al., 1998; Lindberg and Amsterdam, 2008). On the contrary, episodic excessive alcohol consumption or alcohol abuse commonly referred to as “binge drinking” is related to a large number of medical, social and work related problems (negative effects), including the development of alcohol dependence syndrome, several chronic diseases (like liver cirrhosis, cardiomyopathy, encephalopathies, polyneuropathy, dementia), accidents, violent behavior as well as suicide which eventually lead to death (Estruch et al., 1993; Spies et al., 2001; O’Keefe et al., 2007; Brien et al., 2011; Ronksley et al., 2011). One of the organs most affected by chronic alcohol consumption is the liver; the progression of disease begins with fatty liver, which then develops into steatohepatitis, fibrosis and cirrhosis (Lee and Friedman, 2011). A further complication of chronic alcoholism is the development of hepatocellular carcinoma (HCC) and several other types of cancer like cancer of upper aerodigestive tract, colorectal cancer and female breast cancer (Adachi et al., 1991; Schutze et al., 2011). Reports indicate that even light to moderate levels of alcohol consumption leads to several common cancers, mainly those of the breast (Allen et al., 2009). Alcohol consumption increases circulating levels of estrogen and the production of reactive oxygen species (ROS) during alcohol metabolism, including DNA damage that results in breast cancer (Coronado et al., 2011; Seitz et al., 2012).

Fig. 1.5: Ethanol
In males, chronic alcoholism has been reported to cause serious hepatic disorders resulting in hyper-estrogenization and reduced rate of production of testosterone. The metabolic inactivation of testosterone increases due to increased activity of the enzymes of endoplasmic reticulum of hepatocytes which account for decreased sperm count, sperm maturation, and infertility in males who are chronic alcoholics. Studies confirm that chronic ethanol consumption causes a prolonged and severe decrement in serum growth hormone leading to decreased sperm count and sperm maturity in male rats (Tentler et al., 1997). Acetaldehyde, a metabolic product of ethanol, is also reported to decrease testosterone biosynthesis in the Leydig cells (Kaliszuk et al., 1989).

![Alcohol metabolism diagram](image)

**Fig. I.6: Alcohol metabolism**

Reports indicate that alcohol abuse has a deleterious effect at all levels of male reproductive system. Alcohol interferes in the feedback mechanisms of hypothalamic-pituitary-gonadal (HPG) axis resulting in impairment of production and secretion of adequate quantity of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) leading to deterioration of Sertoli cells (Emanuele and Emanuele, 1998). Alcohol influences the Sertoli cell functions by producing damage to some of the proteins required for sperm cell production (Zhu et al., 1997). Alcohol also affects the Leydig cells and reduces blood levels of testosterone, by reducing its production and by
increasing its metabolic clearance (Gordon et al., 1976). It has been reported that alcohol-induced reduction in levels of testosterone, LH and FSH not only hampers their normal morphological development and maturation of spermatozoa (producing significant teratozoospermia), but also slows down sperm production by testicular germ cells (oligozoospermia), and sometimes even lead to azoospermia especially in heavy alcoholics (Emanuele and Emanuele, 1998; Guthauser et al., 2014). Studies indicate partial to complete spermatogenic arrest amongst moderate to heavy alcohol consumers, even leading to “sertoli cell only” syndrome in advanced cases, indicating severe testicular damage (Pajarinen et al., 1996; Villatta et al., 1997). Progressive damage to testes and reduction of sex hormones leads to loss of secondary sexual characteristics and development of impotence and infertility (Emanuele and Emanuele, 1998).

However, many workers at different times have reported their results of investigation on the effects of various drugs at clinical and subclinical doses and their role in different metabolic pathways but the reports of investigation on complicated mesh of relationship among the types of drugs, dosage of drugs, their differential effect on the process of spermatogenesis, hormone profile and drug-induced stress are still fragmentary.
Aim and Objectives:

The present study is aimed to investigate the effect of certain habit-forming and anabolic drugs on fertility status in male mice and to explore the interrelation among sperm count, reproductive hormone concentration, oxidative stress and structural alteration in the male gonadal tissue.

The study is designed with the following objectives:

➢ To assess the fertility status by monitoring the hormonal changes in experimental condition.
➢ To explore the impact of oxidative stress on fertility status.
➢ To assess the changes in sperm concentration in drug abused experimental condition.
➢ To determine the structural alteration of male gonadal tissue.