Substance abuse, also known as drug abuse, a patterned use of a substance (drug) in which the user consumes the substance in amounts harmful to themselves or others, does not exclude dependency but is otherwise used in a similar manner in non-medical contexts. It has a huge range of definitions related to taking a psychoactive or performance enhancing drug for a non-therapeutic or non-medical effect. It is a condition in which prescription medication with sedative, anxiolytic, analgesic or stimulant properties are used for mood alteration or intoxication ignoring the fact that overdose of such medicines have serious adverse effects.

Drug addiction is a complex, chronic, often relapsing brain disease, characterized by compulsive drug seeking and use, despite harmful consequences to the drug addict and those around them. While each drug produces different physical effects, repeated use of substances alter the way the brain looks and functions.

Illicit drug use, which is prevalent in our society, may adversely impact male fertility. Use of these illicit substances is often during reproductive years or during critical periods of testicular development. Illicit drug use affects the hormonal axis and causes impairments in semen analysis and functional sperm parameters. Exposures to certain drugs and toxins may play a role in male infertility. Infertility can be the result of reproductive issues caused by the use of recreational and prescription drugs. Drugs including anabolic steroids, alcohol and tobacco can have a very negative impact on the reproductive system (Fronczac et al., 2012).

Although estimates have been made regarding the prevalence of illicit drug use, it cannot be determined with accuracy how often illicit drug use leads to impaired spermatogenesis.
In the present study on the effect of certain habit forming and anabolic drugs on fertility status in male mice, the status of hormone profile constituting gonadotropins viz., follicle-stimulating hormone (FSH) and luteinizing hormone (LH), testosterone, estradiol and prolactin (PRL) is monitored on administration of three recreational drugs with a simultaneous investigation on the oxidative stress markers. The lipid peroxides and protein peroxides are selected as representative parameters for determination of oxidative stress. In order to support the biochemical changes, histopathological study of testes tissue with measurement of sperm concentration (sperm count) has also been conducted. The drugs selected for the study are anabolic-androgenic steroid (AAS), alcohol and nicotine. In case of anabolic androgenic steroid (AAS)-treated group, 0.1 ml of 2.5 mg of nandrolone decanoate is injected intramuscularly per week; the effect of alcohol is studied by daily administration of 15 ml of 10% ethanol orally and the impact of nicotine is observed by daily oral administration in a series of three increasing doses as 120 µg, 240 µg and 480 µg in the 1st, 2nd and 3rd month respectively during the experimental period of 90 days / 3 months.

The present investigation shows an overall scenario of decreased serum FSH values in all the experimental groups throughout the experimental period, however, the picture depicts some amount of differences in deviation from normal base line in the three different experimental groups. Though in AAS- treated group, a decreased value of serum FSH is recorded from the initial period of 10th day to the terminal part of the experiment, however, the decrease is observed to be only apparent up to 30th day (p>0.05), on 45th day it is found to be significant (p<0.05) and 60th day onwards the deviation is highly significant (p<0.01). In alcohol-treated group, minimal decrease is observed up to 60th day with 5.63% deviation from the normal base line but significant
decrease (p<0.05) with 13.57% deviation from the normal base line (Fig. IV.1) is observed on the terminal part of the experiment. Similar trend of decreased serum FSH is also observed in nicotine-treated group which is only apparent up to 45th day (p>0.05) and 60th day onwards the decrease is found to be highly significant (p<0.01).

Of the three recreational drugs used in the present experimental set up, the AAS is found to be more potent in declining FSH value and after 60th day it becomes 40% below the normal base line. The alcohol administration shows only 13.57% decrease on 90th day whereas nicotine exhibits an intermediate effect by declining 20% below the normal baseline towards the end of the experimental period. However, the initial effect of all the three drugs is found to be similar up to 20th day but 30th day onwards till the terminal part of experiment highly significant decrease (p<0.01) is observed in AAS and nicotine treated groups.

Luteinizing hormone (LH), the other representative member of gonadotropin, exhibits general declining trend in all the experimental groups throughout the experimental period from 10th to 90th day though it is only apparent up to 30th day. The highest amount of deviation (43%) from the normal base line is observed in case of AAS-treated group. However, of the three drugs, alcohol occupies the intermediate position as it is more effective in declining LH than that of nicotine throughout the experimental period which is more pronounced on 90th day as the deviation is about 20% in alcohol-administered group and only 15% in nicotine-treated group (Fig IV.2).

Of the two gonadotropins, LH is found to be more affected than FSH in all the three experimental groups. Though early inhibition i.e., from 20th day, is observed in case of FSH whereas the effect is delayed (marked after 30th day) in case of LH. Both FSH and LH are affected markedly by AAS than nicotine and alcohol. However, nicotine effect
is more pronounced in case of FSH and in case of LH alcohol effect is more than that of nicotine.

Suppression of gonadotropins have been observed on AAS treatment which is consistent with prior literature (Harkness et al., 1975; Clerico et al., 1981; Small et al., 1984; Alen et al., 1985, 1987; Daly et al., 2003). In humans and rats, treatment with anabolic steroids is reported to suppress secretion of FSH and LH (Bijlsma et al., 1982; Grokett et al., 1992). Nagata et al. (1999) has reported decreased FSH and LH concentration in stallions after nandrolone decanoate treatment. This is in contrary to some earlier studies that reported increase in gonadotropin concentration (Aakvaag and Stromme, 1974; Hervey et al., 1976; Remes et al., 1977). The findings of suppressed gonadotropin levels on alcohol administration in the present investigation also support earlier reports (Emanuele and Emanuele, 1998).

Testosterone, the principal male gonadal hormone, is taken as a probe parameter to assess male infertility in the present experimental set-up. The results obtained depict a clear picture of inhibition with decreased value of testosterone in all the three experimental groups throughout the experimental period of 90 days. The hormone level is influenced from the initiation of the experiment i.e. on 10th day, on AAS and alcohol administration which, however, is delayed in case of nicotine treatment as it is observed from 30th day but in the terminal part of the experiment nicotine effect is more pronounced than that of alcohol. Highest amount of decrease is observed in AAS-treated group which is about 48% decline from the normal base line. In AAS-treated group, the decrease is found to be significant (p<0.01) throughout the experimental period i.e., from 10th to 90th day whereas in alcohol and nicotine treated group, the
decrease is found to be only apparent up to 45\textsuperscript{th} day (p>0.05) which has become significantly decreased (p<0.05 and p<0.01) after 60\textsuperscript{th} day (Fig IV.3).

The findings of decreased testosterone concentration on administration of recreational drugs in the present study are in conformity with some previous reports (Harkness \textit{et al.}, 1975; Gordon \textit{et al.}, 1976; Schurmeyer \textit{et al.}, 1984; Small \textit{et al.}, 1984; Ruokonen \textit{et al.}, 1985; Alen \textit{et al.}, 1987; Jarow and Lipshultz, 1990; Daly \textit{et al.}, 2003; Shchelochkov \textit{et al.}, 2012).

For a long time, estrogens in the human male have been regarded as a mere by-product of testosterone synthesis. Recently, with the development of molecular biology, Faustini-Fustini \textit{et al.} (1999) and Rochira \textit{et al.} (2005) suggested that estrogens regulate several functions in men, including human reproduction. Nowadays, the presence of estrogens in the human testes is well documented, and all previous studies definitely changed some classical standpoints in endocrinology, providing evidence that estrogens also exert a wide range of biological effects in men and not only in women (Sharpe, 1998; Deroo and Korach, 2006; Carreau \textit{et al.}, 2012). Estrogens play an important role in pituitary-gonadal interactions in men (Smith \textit{et al.}, 1994). In the testes, estrogen is reported to be essential for male germ cell development. It has been estimated that the testes can account for 15\% of circulating estrogens and hence, local production of estrogens, both intratesticular and extragonadal, is of physiologic significance throughout adult life in males (Hemsell \textit{et al.}, 1974).

In the present study, serum estradiol is estimated as a representative hormone of estrogen for assessment of influence of estradiol in male fertility status. It is clearly evident that anabolic-androgenic steroid (AAS) and alcohol enhance the hormone level up to 5.14\% and 16.4\% on 90\textsuperscript{th} day of the experiment respectively, whereas in nicotine-
administered group the hormone level is decreased after 30th day which is more pronounced on 90th day i.e., the terminal part of the experiment. The effect of all the three drugs on serum estradiol level is of uniform gradient up to 20th day of treatment which is exhibited by insignificant increase (p>0.05) of 3.63%, 3.63% and 1.37% deviation from the normal base line in case of AAS, alcohol and nicotine treatment respectively (Fig IV.4).

The findings of elevated estradiol level by recreational drugs in the present investigation supports the earlier reports of elevation of estradiol by alcohol (Kaliszuk et al., 1989) and anabolic-androgenic steroids (Alen et al., 1985, 1987).

In the present investigation, AAS and alcohol exhibit similar trend of increased value of estradiol but nicotine exerts an opposite result of about 11% decrease from 45th day onwards up to 90th day of experiment. Of the two drugs – anabolic androgenic steroid (AAS) and alcohol, alcohol is found to be more effective in accelerating the hormone level than that of anabolic-androgenic steroid (AAS) which is 16.4% and 5.14% from the baseline respectively (Fig IV.4).

Normal serum concentrations of prolactin (PRL) have been reported to exert permissive roles in the male reproductive tract, but excessive serum prolactin concentration has been correlated with infertility, hypogonadism and impotence (Segal et al., 1979; Gonzales et al., 1989). The trend of Prolactin under different drug abuse conditions of present study indicates alcohol as a marked inducer which increases the prolactin level up to 14% from the baseline at the end of the experimental period. A steady and gradual increase is observed up to 60th day which, however, is only apparent (p>0.05) and thereafter a significant increase (p<0.05) is recorded. It is also noticed that prolactin (PRL) is less responsive to anabolic-androgenic steroid (AAS) with insignificant
decrease (p>0.05) of 0.76 to 5% deviation from the normal base line throughout the experiment. Though nicotine is found to be an early inducer of prolactin, after 30th day the status is changed abruptly to a declining trend and on 75th and 90th day the deviation is observed to be 17 and 16% below the normal baseline respectively (Fig IV.5).

The observation of the present study of depressing effect of nicotine on prolactin can be correlated to the earlier suggestions (Corrigall et al., 1992; Rose and Corrigall, 1997; Di Chiara, 2000; Watkins et al., 2000) that nicotine triggers increased extracellular dopamine levels which has prolactin-inhibitory effect.

The overall scenario of the hormonal status in the present experimental condition is that anabolic-androgenic steroid (AAS) has a depressing effect on FSH, LH, testosterone and prolactin. Of these four hormones, PRL is found to be minimally affected (-1.56 deviation) whereas the most affected hormone is testosterone followed by FSH and LH (Fig. V.I). An enhanced effect of AAS is observed in case of estradiol but it is only 5.14% above the normal base line on 90th day of experiment.

**Fig V.1:** Presenting the effect of anabolic androgenic steroid (AAS) treatment on hormonal status.
In the present study set-up, alcohol is found to be inducer of estradiol and prolactin, which, however depresses testosterone followed by LH and FSH (Fig V.2). But the picture of nicotine effect is completely inhibitory for all the five hormones taken as study parameters in the present experiment. The highest amount of inhibition is observed in case of testosterone similar to that recorded in case of AAS and alcohol. The second most affected hormone by nicotine treatment is FSH followed by prolactin, LH and estradiol (Fig V.3).

Fig V.2: Presenting the effect of alcohol treatment on hormonal status.
Fig V.3: Presenting the effect of nicotine treatment on hormonal status.

Human body always maintains a very low steady-state concentration of Reactive oxygen species (ROS) by its defense mechanism but when the production of free radicals increases they may overcome the scavenging capacity of the antioxidant system, resulting in an “oxidative stress” and damage to biological targets. The stress may be amplified and propagated by an autocatalytic cycle of metabolic stress, tissue damage and cell death, leading to further increase in free radical production and depletion of antioxidants (Baynes, 1991). Oxidative stress seems to play an important role in the development and pathogenesis of various disorders including spermatogenesis.

Oxidative stress has been identified to play a key role in the pathogenesis of subfertility in both males and females. Recent advances in the field of reproductive medicine have focused the attention of many researchers to consider reactive oxygen species (ROS) as one of the mediators of infertility causing sperm dysfunction. Although, ROS is
involved in many physiological functions of human spermatozoa, their excess production results in oxidative stress (Agarwal et al., 2005). Reports indicate that low levels of ROS are necessary to optimize the maturation and function of spermatozoa whereas inappropriately high levels of ROS produced by spermatozoa trigger lipid peroxidation, which damages the sperm’s plasma membrane and cause oxidative stress, thus impairing their capacity to fertilize (Cummins et al., 1994; Ruder et al., 2009).

Since little information is available on the effects of 17-β-alkylated steroid treatment on oxidative stress markers, we aimed to investigate whether treatment of mice with Nandrolone decanoate modified oxidative stress markers through studying TBARS production as a result of lipid peroxidation. Fig V.4 shows that administration of Nandrolone decanoate induced a significant increase (p<0.01) in TBARS levels which serves as an index of extended lipid peroxidation. To our knowledge, this is the second time that the effects of Nandrolone decanoate treatment on oxidative stress biomarker levels have been studied. AAS seek to maximize the anabolic effects and overcome the catabolic pathways thus increasing anabolic pathways, so the possibility of oxidative stress condition could increase (Saborido et al., 1993; Molano et al., 1997).

Change of lipid peroxide content in different tissues in the present experimental set-up is monitored in three principal tissues as blood (the circulating fluid), liver (the principal metabolic centre) and testes (the target reproductive tissue). The present study clearly reveals that the trend of increase in lipid peroxide content is similar in all the three tissues, blood, liver and testes. The increase is found to be sustained till the end of the experiment i.e., up to 90th day which is about 171% in blood, 110% in liver and 152% in testes tissue on AAS administration (Fig V.4). The range of increase after alcohol treatment is found to be 43 to 199% in blood, 111 to 158% in liver and 14 to 108% in
testes tissue (Fig V.5) but in case of nicotine, though the intensity of initial increase is not so significant, the highest amount of increase in LPO is observed in blood with a wide range of 28-256%. In liver and testes tissue, the range of deviation is 8-99% and 7-63% above the base line respectively (Fig V.6).

Fig. V.4: Presenting the effect of anabolic androgenic steroid (AAS) treatment on lipid peroxide (LPO) content in blood, liver and testes tissues.

Fig. V.5: Presenting the effect of alcohol treatment on lipid peroxide (LPO) content in blood, liver and testes tissues.
Gradual increase in lipid peroxide is observed in all the tissues with three different drug administrations but differential increase is found in the three different tissues. In case of blood, ultimate increase is more pronounced in case of nicotine followed by alcohol and AAS treatment. Though the gradient of increase is almost uniform after AAS administration, however, initial steep increase is observed which is from 85-142% from normal base line.

Liver tissue exhibits highest amount of lipid peroxides in case of alcohol treatment (158.40%) and lowest amount in case of nicotine administration (99.60%). On AAS treatment, the content of lipid peroxides is found to be intermediate (Fig IV.7). Though similar trend is observed on administration of all the three drugs, the intensity of increase is different which is only 9%, 47% and 111% above the normal base line in case of nicotine, AAS and alcohol treatment respectively. The deviation is found to be highly significant (p<0.01) in both AAS and alcohol-treated groups throughout the
experimental period whereas the deviation is found to be highly significant (p<0.01) only after 60\textsuperscript{th} day of nicotine treatment.

In testes tissue, AAS is more potent in exerting an inducing effect at the terminal part of the experiment i.e., on 90\textsuperscript{th} day and minimal effect is exerted by nicotine which is similar to blood and liver.

The evaluation of protein peroxide, the other marker of oxidative stress, reveals similar and parallel increasing trend is all the three tissues in all the experimental groups. The magnitude of increase, however, is different in different tissues. The highest amount of protein peroxides is found in circulating fluid as compared to liver and testes tissues in all the groups. Of the three drugs, alcohol is found to be more effective in enhancing the serum protein peroxide than the other two drugs which is marked as 143 to 293\%, 100 to 181\% and 71 to 267\% deviation from the normal base line in alcohol, AAS and nicotine – treated groups respectively.

The general trend of protein peroxide in AAS- treated group is similar in both serum and liver which is noted as gradual increase in serum protein peroxide up to 60\textsuperscript{th} day to 90\textsuperscript{th} day of experiment in case of liver tissue.

![Graph](image-url)

**Fig. V.7:** Presenting the effect of anabolic androgenic steroid (AAS) treatment on protein peroxide (PPO) content in blood, liver and testes tissues.
The overall and generalized effect of the recreational drugs of the present study on oxidative stress markers (LPO and PPO) can be summarized as - alcohol as the potent
inducer, blood as the most affected tissue and nicotine as the delayed enhancer of lipid and protein peroxides.

The observation as tabulated and represented in the present study supports the investigation of the previous workers (Kovacic, 2005; Toda and Ayajiki, 2010). The relationship between consumption of recreational drugs and male infertility remains controversial. A number of studies have shown that chronic use of these drugs detrimentally affects sperm concentration, motility and DNA damage (Pattersen et al., 1990; Yamamoto et al., 1998; Hassan et al., 2009). However, no association between drug abuse and sperm quality has also been reported by some other workers (Vogt et al., 1986; Dikshit et al., 1987; Lewin et al., 1991).

In the present study, sperm concentration (or sperm count) taken as a biomarker for assessment of male fertility status is found to be highly affected on chronic use of the drugs viz., AAS, alcohol and nicotine. A gradual and highly significant decrease (p<0.01) is observed in all the three groups and in case of AAS-treated group, the declining effect is more pronounced than that of alcohol and nicotine. Of the three drugs, the effect of alcohol is found to be significant (P<0.05) on 90th day only, a significant decrease (p<0.05) is observed on 60th and 90th day in case of nicotine whereas AAS treated group exhibits significant decrease from 30th day onwards up to the terminal part of the experiment. The findings of the present investigation are in conformity with the previous reports (Kaliszuk et al., 1989; Tentler et al., 1997). The observations of decreased sperm count in the present study after administration of the three drugs can be correlated with histological changes observed in the testis tissue. AAS is found to be the most potent drug causing maximum alterations in the
histological architecture of the testis tissue followed by nicotine and alcohol. (Figures IV.14, IV.16, IV.18, IV.20, IV.22, IV.24, IV.26, IV.28, IV.30 and IV.32).

The correlation analysis of the results obtained in the present investigation reveals significant correlation ($r>0.5$) between sperm count and hormone profile in general and oxidative stress markers. However, correlation between sperm count and individual hormones is different with different drug administration as in case of AAS treatment, the correlation with PRL is insignificant ($r= 0.205$) as indicated in the Table V.1 and Figures V.10, V.11, V.12, V.13, V.14, V.15, V.16, V.17, V.18, V.19.

Table V.1: Presenting correlation ($r$) between different experimental parameters in different experimental groups.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CORRELATION(r value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AAS-treated group</td>
</tr>
<tr>
<td>FSH- Sperm Count</td>
<td>1.000</td>
</tr>
<tr>
<td>LH- Sperm Count</td>
<td>0.670</td>
</tr>
<tr>
<td>Testosterone- Sperm Count</td>
<td>0.913</td>
</tr>
<tr>
<td>Estradiol- Sperm Count</td>
<td>0.986</td>
</tr>
<tr>
<td>Prolactin- Sperm Count</td>
<td>0.205</td>
</tr>
<tr>
<td>LPO blood- Sperm Count</td>
<td>0.202</td>
</tr>
<tr>
<td>LPO liver- Sperm Count</td>
<td>0.674</td>
</tr>
<tr>
<td>LPO testes- Sperm Count</td>
<td>0.653</td>
</tr>
<tr>
<td>PPO blood- Sperm Count</td>
<td>0.170</td>
</tr>
<tr>
<td>PPO liver- Sperm Count</td>
<td>0.999</td>
</tr>
<tr>
<td>PPO testes- Sperm Count</td>
<td>0.752</td>
</tr>
</tbody>
</table>
Fig V.10: Graphical representation showing interrelation between FSH and Sperm Count.

Fig V.11: Graphical representation showing interrelation between LH and Sperm Count.
Fig V.12: Graphical representation showing interrelation between Testosterone and Sperm Count.

Fig V.13: Graphical representation showing interrelation between Estradiol and Sperm Count.
Fig V.14: Graphical representation showing interrelation between Prolactin and Sperm Count.

Fig V.15: Graphical representation showing interrelation between Blood LPO and Sperm Count.
Fig V.16: Graphical representation showing interrelation between Liver LPO and Sperm Count.

Fig V.17: Graphical representation showing interrelation between Testes LPO and Sperm Count.
Fig V.18: Graphical representation showing interrelation between Blood PPO and Sperm Count.

Fig V.19: Graphical representation showing interrelation between Liver PPO and Sperm Count.
On a concerted observation over the trends of adjustment with changing sperm count, hormone profile and oxidative stress markers under the present experimental set-up with AAS, alcohol and nicotine administration, it has come out with reasonable clarity about the relationship and metabolic readjustment. It is fully supportive by the present findings that there is a total suppression of hormone profile in all the three drug-administered groups except in AAS and alcohol where estradiol and prolactin is found to be above normal base line. Under the prevailing metabolic states of the current experimental set-up with increased amount of oxidative stress markers, all the three tissues support the authenticity of the drugs as oxidative stress enhancer.

The trend of oxidative stress markers (LPO and PPO) under different conditions of the present study indicates alcohol as a general inducer in liver and testes tissue though some amount of tissue differentiation is exhibited by different drug administration.
The present study probing the role of habit-forming (alcohol and nicotine) and anabolic drugs (nandrolone decanoate) on fertility status is undertaken with two gonadotropins (FSH and LH), two gonadal hormones (testosterone and estradiol) and prolactin (PRL); two oxidative stress markers (LPO and PPO) and measurement of sperm concentration. The alteration of these components in the total metabolism of the experimental system is presented as individual entity with drawing some simultaneous correlation above relevant situations (Figures V.10, V.11, V.12, V.13, V.14, V.15, V.16, V.17, V.18 and V.19). With an attempt to have summarized projection of the alterations scenario under the present experimental set up in the form of the extent of deviation from the normal metabolic state, all the fractional observations are graphically presented in a unified form in Fig V.21, Fig V.22 and Fig V.23.

![Graph](image)

**Fig. V.21:** Overall scenario of the hormone profile, oxidative stress markers and sperm count on AAS administration.
In conclusion, the present study provides a clear evidence for the suppression of sperm count and hormone profile except solitary hormone, estradiol, and accelerated oxidative stress marked by increase in LPO and PPO content in different tissues on administration of all the three recreational drugs which is supported by structural alteration of male gonadal tissue indicated by hypospermatogenesis, maturation arrest, decrease in the density of spermatozoa, spermatids, primary spermatocytes and spermatogonia, degenerative changes at certain regions of the testes tissue and dystrophic calcification in some of the seminiferous tubules.