Effect of Chronic Use of Recreational Drugs on the Sperm Count in Albino Mice

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Abstract

Chronic use of various recreational drugs is leading to drug abuse which is increasingly developed in the modern society among the various populations throughout the world. The present study was conducted to investigate the impact of a few recreational drugs viz., anabolic androgenic steroids (AAS), alcohol and nicotine on male fertility status which was assessed by measuring sperm count in male albino mice. Semen samples were collected from normal, AAS-treated, alcohol-treated and nicotine-treated mice and the sperm count (in millions/ml semen) was assessed at regular intervals i.e. on 10\textsuperscript{th}, 20\textsuperscript{th}, 30\textsuperscript{th}, 45\textsuperscript{th}, 60\textsuperscript{th}, 75\textsuperscript{th} and 90\textsuperscript{th} day of treatment during the experimental period of 90 days. The results showed a significant decrease in the sperm count in AAS-treated, nicotine-treated (p<0.01) and alcohol-treated mice (p<0.05) compared to that of the normal mice. The present study clearly indicates suppression of sperm count on AAS, alcohol and nicotine treatment which may be one of the contributing factors in male infertility.

\textbf{Keywords:} AAS; Alcohol; Drug abuse; Nicotine; Recreational Drugs; Sperm count.
1. Introduction

Infertility, a critical component of reproductive health, is a major public health problem in the modern society. It affects 10-15% of couples across the globe [1]. It can lead to distress and depression, as well as discrimination and ostracism among married couples [2,3]. Male factor infertility is considered to be the sole contributing factor in conception difficulties of up to 40% of infertile couples [4]. 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 [5-8]. Although in some men a specific disorder may be present, in majority of them no apparent reason for infertility has been reported. In the present day scenario, lifestyle factors like recreational drug abuse 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. [9].

The abuse of AAS is under constant debate worldwide. 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 [10-18]. One of the most pronounced effects of AAS abuse is its negative impact on the hypothalamic-pituitary-gonadal axis [19]. AAS via negative feedback to hypothalamus induce hypogonadotrophic-hypogonadism associated with decreased serum testosterone concentrations, testicular atrophy, impaired steroidogenesis and spermatogenesis [20-22]. AAS abuse leads to marked depression of serum testosterone, sex hormone-binding globulin (SHBG) as well as gonadotrophins i.e., luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [23,24].

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 [25]. 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 [26]. A number of studies have shown that long term chronic intake of nicotine and alcohol 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 [27-29].

However, many workers at different times have reported their results of investigation on 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 and their differential effect on the process of spermatogenesis are still fragmentary. Therefore, the aim of the present study is to investigate the impact of a few recreational drugs viz. anabolic androgenic steroids (AAS), alcohol and nicotine on male fertility status in albino mice.
2. Materials and Methods

2.1 Animals

One hundred and forty (140) adult male albino mice, weighing between 20-25 g (three to four months old), were randomly selected from the animal house of the Zoology Department of Gauhati University, Guwahati, Assam (India) after approval of the Ethical Committee of Animal Welfare of Gauhati University. Before starting the experimental procedure, all the animals were acclimatized in the animal room for four weeks and fed on standard animal diet. Adequate measures were taken to minimize pain or discomfort to the mice and the experiments were conducted in accordance with international standards on animal welfare and were also compliant with local and national regulations.

2.2 Animal Grouping and Treatment

The 140 animals were randomly divided into four different groups of 35 each as follows:

**Group-I (Normal Group)**, receiving normal standard diet but did not receive any treatment;

**Group-II (AAS-treated Group)**, receiving intramuscular injection of 0.1ml of 2.5 mg of nandrolone decanoate weekly during the experimental period of 13 weeks. In the present study, a dose of 2.5 mg Nandrolone decanoate is chosen only to ascertain the effect in this particular dose. Doses were selected as 100mg/kg body weight [30,31].

**Group-III (Alcohol-treated Group)**, receiving 15 ml of 10% ethanol per day orally during the experimental period of 90 days.

**Group – IV (Nicotine-treated Group)**, receiving oral administration of 120 µg nicotine per day from 1st day to 30th day, 240 µg nicotine per day from 31st day to 60th day and 480 µg nicotine per day from 61st day to 90th day during the experimental period of 90 days. Nicotine is administered orally in a series of three doses, viz. 4,8 and 16 mg/kg body weight respectively [32].

2.3 Semen Collection, Preparation of spermatozoa and Estimation of Sperm Count

The male albino mice of normal and different experimental groups were sacrificed on 10th, 20th, 30th, 45th, 60th, 75th and 90th day of treatment for collection of semen samples. The animals were anaesthetized with diethyl ether by using an anaesthetic mask and a “T” incision is made from pubic symphysis to the upper abdomen for exposing the abdominal region. The testis, epididymis, seminal vesicle, prostate gland and vas deferens were dissected out immediately and separated from the adherent tissue. Caudae epididymidis and vasa deferentia were excised and rinsed with medium containing 150 mM NaCl, 5 mM KCl, 2mM CaCl₂, 1 mM MgCl₂, 30 mM HEPES, 10 mM glucose, 10 mM lactic acid, and 1 mM pyruvic acid (pH 7.4) which
were then transferred to 1 ml of medium supplemented with 5 mg of bovine serum albumin per ml and 15 mM NaHCO₃. Semen was allowed to exude (15 min at 37°C, 5% CO₂) from three to five small incisions. Cells were diluted to 4 ml and collected twice by sedimentation (400 g; 5 min) [33]. The sperm count was measured using Neubaeur chamber [34].

2.4 Statistical Analysis

The results obtained were statistically analyzed for t-test, percentage deviation, coefficient of variation (CV) and others following Croxton [35].

3. Results

The obtained results were summarized in Table 1. The data were presented as mean ± SEM. The analysis revealed decrease in the sperm count in AAS-treated, alcohol-treated as well as nicotine-treated mice compared to that of the normal mice.

Table 1. Presenting the mean values of sperm count (in millions/ml) of different groups at different days interval.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DAYS OF TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10th</td>
</tr>
<tr>
<td>GROUP-I (NORMAL GROUP)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>44.46±0.99</td>
</tr>
<tr>
<td>GROUP-II (AAS-TREATED GROUP)</td>
<td>42.35± 1.72*</td>
</tr>
<tr>
<td>GROUP-III (ALCOHOL-TREATED GROUP)</td>
<td>44.23± 1.95*</td>
</tr>
<tr>
<td>GROUP-IV (NICOTINE-TREATED GROUP)</td>
<td>43.81± 2.79*</td>
</tr>
</tbody>
</table>

Mean values are expressed as mean ± SEM.
In the group of animals administered with anabolic androgenic steroid (AAS), the mean values of sperm count were found to be in decreasing trend which were recorded as 42.35 ± 1.72 millions/ml on 10\textsuperscript{th} day to 30 ± 1.23 millions/ml on 90\textsuperscript{th} day of treatment.

In the alcohol treated group of animals, the mean values of sperm count were found to be in decreasing trend as 44.23 ± 1.95 on 10\textsuperscript{th} day to 37.25 ± 2.78 millions/ml on 90\textsuperscript{th} day of treatment.

In the group of animals treated with nicotine, the mean values of sperm count were found to be in decreasing trend in the range of 43.81 ± 2.79 to 34.72 ± 2.21 millions /ml during the experimental period of 90 days.

![Graph showing percentage deviation of sperm count (in millions/ml) of different experimental groups from mean value of normal group.](image)

**Fig 1.** Presenting the percentage deviation of sperm count (in millions/ml) of different experimental groups from mean value of normal group.

4. **Discussion**

In the present investigation, with the experimental dosage (2.5 mg/week) of nandrolone decanoate, a highly significant decrease (p<0.01) in the sperm count (in millions/ml semen) was observed during the experimental period of 90 days. However, decrease in sperm count was found to be insignificant upto 20\textsuperscript{th} day, significant on 30\textsuperscript{th} day and highly significant from 45\textsuperscript{th} day onwards which was sustained upto the
terminal part of the experimental period (Table 1). On 90th day of treatment, the mean value was found to be lowest with 32.09% declination from the normal base line (Fig. 1).

In case of alcohol-treated group, with the experimental dosage of alcohol (15 ml of 10% ethanol), a significant decrease (p<0.05) in the sperm count (in millions/ml semen) was recorded during the experimental period of 90 days. The decline in the sperm count was found to be insignificant upto 30th day whereas it was observed to be significant on 45th, 75th and 90th day of treatment (Table 1) with 16.33% declination from the normal base line on 90th day (Fig. 1).

In the nicotine-treated group of mice, the decline in sperm count was found to be insignificant upto 45th day of treatment which gradually declined towards the end of the experimental period with highest amount of declination (22.01%) from the normal base line on 90th day (Fig. 1). This reduction in sperm count was observed to be positively correlated with the duration of treatment (Table 1).

The present investigation clearly depicts early declination of sperm count i.e., after 20th day in case AAS treatment followed by alcohol i.e., after 30th day and finally nicotine i.e., after 45th day of treatment (Table 1). Though delayed impact was observed in case of nicotine treatment, the effect was found to be more pronounced in case of nicotine i.e., 22.01% than that of alcohol i.e., 16.33% towards the end of the experimental set-up (Fig. 1). However, maximum declination i.e., 32.09% below normal base line was recorded in case of AAS treatment (Fig. 1).

Thus, the findings of the present investigation are in conformity with the previous reports and clearly indicates suppression of sperm count on AAS, alcohol and nicotine treatment which may impair the fertility status. However, further investigation is needed to correlate the effect of recreational drugs with male infertility.

5. Conclusion

It can be concluded from the present investigation conducted for a period of 90 days that sperm count is maximally suppressed on chronic administration of AAS, followed by nicotine and alcohol respectively. However, further study for longer duration is required in order to support the authenticity of the results.

References


