List of Publications/ Presentations


Effect of in utero exposure to panmasala on pregnancy outcome in Swiss albino mice. Archana K, AK Gautam, Sunil Kumar. Int. conf. on Reproductive Biology and Comparative Endocrinology. Hyderabad, 60. Jan ’09

Effect of panmasala on reproductive performance in Swiss albino mice. Archana K. et al. Int. conf. on Genomics and Biomedical research. Ahmedabad, 55. Jan’09

Germ cell mutagenic potential of Panmasala in male Swiss albino mice. Archana K et al National Seminar on Teratology and Genetic Disorders, BHU, Varanasi, 18, Feb’08. (Awarded BEST PAPER presentation).


Reproductive toxicity of panmasala in male Swiss albino mice. Sunil Kumar, Archana K et al. XXVII Annual conf. of STOX, India, Bangalore, 54, Oct’07.

Demonstration of Panmasala induced deterioration of sperm morphology in Swiss albino mice. Kumar S, Gautam AK, Mojidra BN, Archana K et al. Int. conf. on Toxicology, Toxicogenomics & Occupational Health, Gwalior, 130, Oct’06.
Evaluation of genotoxicity of pan masala employing chromosomal aberration and micronucleus assay in bone marrow cells of the mice

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Abstract
Pan masala is commonly consumed in south-east Asian and other oriental countries as an alternate of tobacco chewing and smoking. Genotoxic potential of pan masala (pan masala plain and pan masala with tobacco known as Gutkha) was evaluated employing chromosome aberration (CA) and micronucleus (MN) assay in vivo. Animals were exposed to three different doses (0.5%, 1.5% and 3%) of pan masala plain (PMP) and gutkha (PMT) through feed for a period of 6 months and micronucleus and chromosomal aberrations were studied in the bone marrow cells. Induction of mean micronuclei in polychromatic erythrocytes (MNPCE) and normochromatic erythrocyte (MNNCE) was higher in both types of pan masala–treated groups with respect to control group. Both pan masala plain and gutkha treatment significantly induced the frequency of MNPCE and MNNCE in the bone marrow cells, indicating the genotoxic potential. Further, slight decline in the ratio of polychromatic erythrocytes to normochromatic erythrocytes was also noticed, suggesting the cytotoxic potential even though the ratio was statistically non significant. A dose-dependent, significant increase in chromosome aberration was observed in both types of pan masala–treated mouse with respect to control. However, no significant difference in micronucleus and chromosomal aberration induction was noticed between two types of pan masala–exposed (PMP and PMT) groups. Results suggest that both types of pan masala, i.e. plain and gutkha, have genotoxic potential.

Keywords
Pan masala, gutkha, genotoxicity, chromosome aberration, micronucleus

Introduction
Pan masala is a dry chewing mixture of areca nut, catechu, lime, cardamom, menthol, spices and unspecified flavouring agents and pan masala-containing tobacco is commonly known as gutkha. Pan masala is commonly consumed in south-east Asian and other oriental countries and also in the some parts of Africa, North America and UK as an alternate of tobacco chewing and smoking. Pan masala and gutkha are marketed under various brand names and the manner in which these products are being advertised has created a high degree of social acceptance. Pan masala became very popular in all the sections of societies irrespective of age and sex. The popularity of pan masala has increased several folds due to the packaging revolution that made these products non-perishable, convenient to carry and use; as a result, the tobacco products, i.e. gutkha, have become a part of the life style as an alternate form of tobacco smoking and chewing. Although the pan masala constitutes areca nut and tobacco, reported to be cyto- and genotoxic, it is being consumed under the misconception of being safer alternative of tobacco chewing. In India, about 13%-50% school and college students chew pan

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Asalas and gutkha on a regular basis (Gupta and Ray, 003). Studies conducted among migrated Asian ethnic group in UK indicated that pan masala chewing habits were also prevalent among children (Farrand et al., 001). Both areca nut and tobacco, the major constituents of pan masala gutkha, are having addictive potential (Winstock, 2002). Hence, consumers of these products get addicted soon to the mixture containing these components.

Kumar and Saiyed (1999) reviewed the data on tobacco and areca nut and reported that these products have mutagenic and/or carcinogenic potential. In addition to the local effects on the oral and oesophageal mucosa, these substances may likely affect other organ systems also (Kumar et al., 2003; Sarma et al., 1992). Areca nut constitutes about 70%-80% of the pan masala mixture and is reported to possess cytotoxic, mutagenic and genotoxic properties (ARC, 2004). Areca nut and its alkaloids are reported to be teratogenic in mice and rats chronically exposed to betel nut or arecoline (Sinha and Rao, 1985). Catechu, another constituent of pan masala (about 10%-15%), has also been reported to possess clastogenic activity (Giri et al., 1988). Therefore, it is worthy to undertake studies on the mixture, i.e. pan masala PMP and gutkha (PMT), that contains some of the components having mutagenic/carcinogenic potential, in order to investigate its genotoxic potential by employing cytogenetic assays i.e. chromosome aberration (CA) and micronucleated (MN) cells, as these tests have been advocated as sensitive and reliable parameters for assessing the genotoxicity (Adler 1984; Norppa and Falck, 2003).

Materials and methods

Animals and treatment

Healthy adult male Swiss albino mice were procured from the National Center of Laboratory Animals, Hyderabad, India, and maintained in the institute’s animal house. A total of 10-12-weeks-old male mice weighing 26 ± 3 g were selected from an inbred colony and maintained under the controlled conditions of temperature 23°C ± 3°C humidity 50% ± 5% and photoperiod (12/12 hour light and dark). The animals were provided with standard mice feed (Amrut feed, Pune, India) and water ad libitum. The animals were housed in a polypropylene cage containing sterile paddy husk as bedding throughout the experiment. Mice were divided in seven groups of six each.

Control group received normal diet, and experimental group were fed 0.5%, 1.5% and 3% of pan masala plain and gutkha through feed, respectively. The doses of pan masala were selected on the basis of food consumption by human and mice to simulate human exposure (habitual chewers chew about 10-12 pouches of 2 g each/day and average human diet is about 1 kg dry weight, hence pan masala consumption is 2% of the diet). One of the commonly used brands of pan masala was used throughout the experiment. The Institutional ethics committee for animal experimentation approved this project work.

Chromosome aberration assay

The mice were sacrificed after 6 months of treatment. Cytogenetic analyses were performed using the modified method of Adler (1984). Animals were injected (intraperitonially [i.p.]) colchicine (10 mg/kg) 2 hours before sacrifice. Bone marrow cells were collected from femur bone in hypotonic solution (0.075 M KCl at 37°C, 5 mL) and incubated at 37°C for 30 min. Bone marrow suspension was centrifuged at 2000 rpm for 10 min, fixed in methanol: acetic acid (Carnoy’s fixative, 3:1 v/v). Centrifugation and fixation were repeated thrice at the intervals of 10 min. The material was resuspended in little volume of fixative, dropped on to chilled micro slides; air dried and stained in 5% buffered Giemsa (pH 6.8). A minimum of 400 metaphases was examined per treatment group.

Micronucleus assay

Schmid’s method (1975) was employed for the micronucleus assay. The bone marrow cells from femur was flushed out with fetal calf serum, mixed and centrifuged at 1000 rpm for 5 min. The pellet was resuspended in a few drops of fetal calf serum. Smears were prepared on pre-cleaned, dry slides, air-dried and fixed in absolute methanol. The slides were stained with May-Grunwald-Giemsa solution. At least 1000 erythrocytes were observed from each animal and the number of polychromatic erythrocytes and normochromatic erythrocytes were evaluated. The micronuclei in them were recorded per 1000 cells and expressed in percentage.

Statistical analysis

Data were presented as mean ± SEM and further subjected to statistical analysis employing Student’s t test for test of significance between control and exposed
Table 1. Effect of pan masala on induction of micronuclei in mouse bone marrow cells

<table>
<thead>
<tr>
<th>Group</th>
<th>% MNPCE</th>
<th>% MNNCE</th>
<th>P/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.42 ± 0.06</td>
<td>0.30 ± 0.08</td>
<td>0.72 ± 0.11</td>
</tr>
<tr>
<td>0.5% PMP</td>
<td>1.14 ± 0.10a</td>
<td>0.40 ± 0.06</td>
<td>0.53 ± 0.04</td>
</tr>
<tr>
<td>1.5% PMP</td>
<td>1.07 ± 0.08b</td>
<td>0.52 ± 0.05c</td>
<td>0.70 ± 0.09</td>
</tr>
<tr>
<td>3.0% PMP</td>
<td>1.66 ± 0.20d</td>
<td>0.74 ± 0.09b</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td>0.5% PMT</td>
<td>1.47 ± 0.13b</td>
<td>0.55 ± 0.09</td>
<td>0.52 ± 0.03</td>
</tr>
<tr>
<td>1.5% PMT</td>
<td>1.32 ± 0.13b</td>
<td>0.57 ± 0.07c</td>
<td>0.54 ± 0.07</td>
</tr>
<tr>
<td>3.0% PMT</td>
<td>1.70 ± 0.07b</td>
<td>0.73 ± 0.05c</td>
<td>0.49 ± 0.05</td>
</tr>
</tbody>
</table>

P/N, ratio of polychromatic erythrocyte to normochromatic erythrocyte; MNPCE, micronuclei in polychromatic erythrocytes; MNNCE, micronuclei in normochromatic erythrocytes; PMP, pan masala plain; and PMT, pan masala gutkha.

a Values are expressed as mean ± SEM.

Table 2 summarizes the data of chromosomal aberrations occurring were mostly in the form of chromosomal gaps, chromatid breaks followed by chromatid gaps and chromosomal breaks (Table 2). Generally, the effect of gutkha was more conspicuous on cytogenetic end points than pan masala plain. However, the effect was found non-significant within the two exposed groups.

Discussion

In the present study, a significant increase in chromosome aberration and micronuclei was observed in the bone marrow cells of mice treated with both the types of pan masala. The effects were more noticeable in groups exposed to higher dose of pan masala than lower dose. The observed effect was slightly higher in PMT-treated group with respect to PMP at higher dose level. The increased frequency of MNPCE ($R^2 = .70$) and MNNCE ($R^2 = .99$) correlated with the different treatment doses of pan masala and found a positive correlation. The same was also true for chromosomal aberrations when a positive correlation ($R^2 = .67$ and .75) was observed with PMP and PMT. The genotoxicity observed in this study can be attributed to the areca nut and tobacco present in the pan masala along with known/unknown other ingredients. Previous studies on various constituents of pan masala documented that arecoline, a major alkaloid of areca nut, was found to increase the frequencies of chromosome aberrations (Panigrahi and Rao, 1982) and micronuclei (Shimame et al., 1984) in mouse bone marrow cells. Further, arecaidine, another alkaloid, induced a dose-dependent increase in the frequency of sister chromatid exchanges in bone marrow cells of Swiss mice (Panigrahi and Rao, 1984). Acute and chronic oral treatment with catechu (Katha), another major component of pan masala, induced significant increase in sister chromatid exchanges in male mice. Katha also induced dominant lethal mutations leading to increased number of post implantation loss in mice (Giri et al., 1988). Polasa et al. (1993) have also reported weak mutagenecity response in Salmonella typhimurium by pan masala. Chromosome-damaging effects of pan masala were also assessed on cultured Chinese hamster ovary cells (Jaju et al., 1992; Patel et al., 1994). The present study, coupled with data available, further emphasized that pan masala have mutagenic and clastogenic potential.
Effect of pan masala on induction of chromosome aberrations in mouse bone marrow cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Chromatid % Gap</th>
<th>Chromatid % Break</th>
<th>Chromosome % Gap</th>
<th>Chromosome % Break</th>
<th>Total % Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.83 ± 0.20</td>
<td>0.42 ± 0.24</td>
<td>-</td>
<td>-</td>
<td>1.25 ± 0.019</td>
</tr>
<tr>
<td>5% PMP</td>
<td>1.07 ± 0.37</td>
<td>1.25 ± 0.40</td>
<td>1.43 ± 0.42</td>
<td>0.18 ± 0.17</td>
<td>3.83 ± 0.74</td>
</tr>
<tr>
<td>5% PMP</td>
<td>0.69 ± 0.37</td>
<td>0.86 ± 0.00</td>
<td>2.41 ± 0.80</td>
<td>0.69 ± 0.37</td>
<td>4.61 ± 0.38</td>
</tr>
<tr>
<td>0.0% PMP</td>
<td>0.81 ± 0.25</td>
<td>1.35 ± 0.25</td>
<td>2.16 ± 0.41</td>
<td>0.54 ± 0.29</td>
<td>5.04 ± 0.74</td>
</tr>
<tr>
<td>5% PMT</td>
<td>0.69 ± 0.22</td>
<td>1.15 ± 0.31</td>
<td>1.85 ± 0.33</td>
<td>-</td>
<td>3.66 ± 0.52</td>
</tr>
<tr>
<td>5% PMT</td>
<td>1.72 ± 0.33</td>
<td>1.03 ± 0.37</td>
<td>1.90 ± 0.75</td>
<td>0.86 ± 0.48</td>
<td>5.48 ± 0.86</td>
</tr>
<tr>
<td>0.0% PMT</td>
<td>1.23 ± 0.31</td>
<td>1.58 ± 0.22</td>
<td>2.46 ± 0.21</td>
<td>0.53 ± 0.22</td>
<td>5.77 ± 0.34</td>
</tr>
</tbody>
</table>

MP, pan masala plain; PMT, pan masala gutkha.
Values are expressed as mean ± SEM.

p < .01.
p < .001.

In the present study, most common chromosomal aberration was chromosome gap followed by chromatid break. Earlier, Deb and Chatterjee (1998) reported that arecoline, an alkaloid of areca nut, induced the chromosome aberration in bone marrow cells of mice, and the most common effect was the chromatid gaps. Adhvaryu et al. (1991) reported that tobacco-areca nut chewers have significantly elevated frequency of chromosome and chromatid aberrations, the majority of which were gaps, were more frequent than chromosome-type aberration. The observed effects i.e. induction of chromosomal aberrations and micronuclei in the present study lend support to the effect of various constituents of areca nut and pan masala.

Tobacco has also been reported to induce the frequency of micronuclei in bone marrow cells of mice (Bhide et al., 1987; Shimame et al., 1984). Trivedi et al. (1993) studied the genotoxic effect of nicotine in combination with arecoline. They observed statistically significant elevation in CA frequency by nicotine and arecoline or in combination showing synergistic effect. The changes were more pronounced in groups exposed to higher dose of nicotine and arecoline and the chromatid gaps were the most frequent abnormality. The observed effects slightly higher in the gutkha-treated group with respect to plain pan masala in the present study might be due to the tobacco present in pan masala gutkha. Harmful effects of pan masala on the cytogenetic end points may be due to the presence of chemicals like arecoline, arecaidine and N-nitrosamines in areca nut, tannin in catechu and also tobacco-specific nitrosamines etc. Earlier, study carried out among pan masala chewers also showed significant increase in micronucleated cells and chromosomal aberrations in the peripheral blood cells as compared to non-chewers (Dave et al., 1991).

Both pan masala plain and gutkha showed adverse effects on cytological parameters, but slightly, except for a higher frequency of abnormalities that were found non-significant within the two exposed groups. This indicates that in addition to tobacco, other ingredients of pan masala, especially areca nut, may be responsible for the induction of micronucleus and chromosomal aberration.

Conclusion
The results affirm that both pan masala plain and gutkha have genotoxic potential as both types of pan masala are found to be potent inducer of chromosomal aberrations and micronuclei in the bone marrow cells of mice. Further studies may be taken on pan masala with shorter duration with lower doses. Based on the genotoxic and carcinogenic potential of pan masala, there is a need for information, education and communication about the adverse effects of pan masala.

Acknowledgements
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References
Adhvaryu SG, Dave BJ, and Trivedi AH (1991) Cytogenetic surveillance of tobacco-areca nut (mava) chewers,
including patients with oral cancers and premalignant conditions. *Mutation Research* 261: 41–49.


Review Article

Lifestyle factors in deteriorating male reproductive health

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Division of Reproductive and Cytotoxicology1, ENVIS-NIOH Centre2, National Institute of Occupational Health (ICMR), Ahmedabad 380 016, India

Many health problems are related to lifestyle and dietary factors. Increasing trend in reproductive disorders observed in recent years may be associated at least in part with these factors, which are compounded by some of the new emergent lifestyles. The data available suggests that lifestyle factors such as obesity, tobacco smoking or chewing, alcohol and some of the illicit drugs like cocaine, cannabis etc and exposure to extreme heat, have adverse effects on male reproduction. The data on other factors such as use of mobile phone and stress on reproductive health are inadequate and need detailed study. Lifestyle related diseases could be lowered with modification in diet, living and working environment etc. Sub-fertile and/or normal subjects have some control over their reproductive function by adopting healthy lifestyles to avoid additional complications.

Keywords: Illicit drugs, Lifestyle factors, Obesity, Reproductive impairment, Semen quality, Stress, Tobacco smoking and chewing

Introduction

There are sufficient evidences of increasing trends in a number of human health problems like cancers, reproductive and developmental defects, cardiovascular problem etc. Environmental, lifestyle, dietary or occupational factors may play an important role behind these trends. Over the last few decades, there have been progressive changes in many aspects of our diet, lifestyle as well as environment. Among lifestyle, factors such as tobacco smoking or chewing, alcohol, caffeine, use of illicit drugs etc. have a profound negative impact on general health. Various health problems like hypertension, diabetes mellitus, high blood cholesterol, obesity, cardiovascular disease and even some types of cancer are related to nutritional as well as lifestyle factors to some extent. These factors may also be responsible to enhance the reproductive disorders in both sexes.

Approximately 15% of the sexually active population is affected by clinical infertility and in 50% cases a male factor is involved, either as a primary problem or in combination with a problem in the female partner1. The cause of deterioration in reproductive health may be attributed to direct or indirect exposure to some of the environmental persistent chemicals, solvents, metals etc. The association of lifestyle factors on deterioration of reproductive health is receiving attention i.e., tobacco smoking and chewing, alcohol, caffeine, high temperature, some dietary components, stress, and some modern electronic gadget have shown to adversely affect reproduction. These factors may impair male fertility by interfering with spermatogenesis, spermiogenesis, motility, sperm DNA and chromatin integrity, hormonal regulation or by reducing the fertilising capacity of spermatozoa. The earlier review published on this aspect are based mainly on single/few life style factors2–5. An attempt has been made to compile the recent data pertaining to male reproductive health with reference to the different lifestyle factors except the data on occupational exposure to chemicals and ionising radiations. The data are summarized in (Table 1).

Obesity and nutritional factors

High caloric foods, sedentary work, no exercise, easy transportation along with increase use of modern technologies that reduce the need for physical activity can explain the increasing prevalence of obesity around the world. Obesity is a condition in which excess body fat gets accumulated and has been associated with an increased risk of many serious illnesses such as cardiovascular diseases, diabetes mellitus and some types of cancer6. It is gradually recognizing that obesity is one of the causes of sub fertility.
Table 1—Lifestyle factors and reproductive impairments in male

<table>
<thead>
<tr>
<th>Factor</th>
<th>Major effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity</td>
<td>• Reduction in semen quality&lt;sup&gt;9,10&lt;/sup&gt;, serum testosterone, SHBG, and inhibin B while elevation in free androgen index and E&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;13-15&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tobacco smoking and chewing</td>
<td>• Alter spermatogenesis&lt;sup&gt;29&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Lower sperm penetration assay scores and greater numbers of leukocytes in the seminal fluid&lt;sup&gt;30,31&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Significantly lower zinc levels&lt;sup&gt;45&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Decrease results of hypoosmotic swelling test&lt;sup&gt;32&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Decrease in anti-oxidant defenses components of the sperm&lt;sup&gt;34-37&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alcohol</td>
<td>• Increase leukocytes in the seminal fluid&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Reduction in seminal quality&lt;sup&gt;58&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Significantly high FSH, LH, and E&lt;sub&gt;2&lt;/sub&gt; and low testosterone and P&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>Stress</td>
<td>• Decrease sperm count, motility and morphology&lt;sup&gt;64&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Involuntary childlessness - higher frequency of male sexual disturbances&lt;sup&gt;65&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heat</td>
<td>• Physical stress - lowers T levels, reduction in LH pulse frequency&lt;sup&gt;67&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Deteriorated sperm morphology, motility and concentration&lt;sup&gt;72&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Increase risk of apoptosis in spermatogenic cells&lt;sup&gt;82&lt;/sup&gt;</td>
</tr>
<tr>
<td>Drugs</td>
<td>• Narcotic drugs decrease gonadotropin and stimulate P secretion&lt;sup&gt;67&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Marijuana - gonadal toxin; increase leukocytes in the seminal fluid&lt;sup&gt;30,38&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Cannabis - reduce progressive sperm motility, acrosome reactions, LH, FSH, T, P, thyroid gland function, and growth hormone while elevate adrenal cortical steroids&lt;sup&gt;89,90&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Khat - semen quality deteriorated, cytoplasmic droplets present&lt;sup&gt;91&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Cocaine decrease in sperm count and motion kinematics (straight line velocity and linearity), linked to abnormal development of their offspring&lt;sup&gt;92-95&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Heroin impaired semen quality, increase circulating total thyroxine, triiodothyronine and decrease P, T, FSH and cortisol&lt;sup&gt;96-99&lt;/sup&gt;</td>
</tr>
<tr>
<td>Radiation-Electromagnetic</td>
<td>• Cell phones - decreases semen quality&lt;sup&gt;103,104&lt;/sup&gt;</td>
</tr>
<tr>
<td>Radiation</td>
<td>• May increase ROS level and decrease in ROS-TAC score leading to oxidative stress&lt;sup&gt;105&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Obesity has been associated with reproductive disorders in women, including menstrual abnormality, infertility, miscarriage, and reduced success of assisted reproduction<sup>74</sup>. Its role in male reproduction has also been documented in recent years. A significant reduction in sperm concentration, and motility was observed in the obese group than in males with BMI < 30 kg/m<sup>2</sup>. Further, in the obese group, sperm count continuously decreased with aging. In addition, men presenting with a BMI > 25 kg/m<sup>2</sup> have fewer chromatin-intact, normal-motile sperm cells per ejaculate<sup>9-10</sup>. Most of the available data suggest negative association between semen quality and obesity. Obesity was also linked with disturbances of penile hemodynamics and found to be an independent clinical factor for vasculogenic erectile dysfunction<sup>11</sup>. But in another study the incidence of erectile dysfunction did not vary across BMI categories when corrected for potential contributing factors<sup>15</sup>, indicating need for further study.

The exact mechanism of obesity-mediated effect on reproduction is not fully understood. However, overweight and obese men were found to have a markedly changed sex hormone profile along with reduced semen quality. Serum testosterone, sex hormone-binding globulin (SHBG), and inhibin B decreased while free androgen index and estradiol (E2) increased with increasing BMI<sup>13-15</sup>. Earlier Strain et al<sup>16</sup>., and Haffner et al<sup>17</sup>., also observed that the plasma levels of SHBG, total testosterone, free testosterone and follicle stimulating hormone (FSH) were lowered in obese men compared to non-obese men. Based on experimental and clinical studies, decline in androgen levels<sup>14,15,18,19</sup>, increased estrogen levels<sup>20</sup> and suppression of the hypothalamic-pituitary-testicular axis<sup>21</sup> have been suggested as potential aetiologies of altered spermatogenesis in obese males<sup>4</sup>.

In addition to obesity, some dietary habits of people from different ethnic background may
have some effect on reproductive health. Chavarro et al. suggested that higher intake of soy foods and soy isoflavones is associated with lower sperm concentration. Kumar et al., observed that smoke had a detrimental effect on sperm function. Sivaswamy et al. detected polycyclic aromatic hydrocarbons (PAHs) in some of the salted, sun dried and oil fried vegetables and fishes. Xia et al. studied four urinary metabolites of PAHs in relation to idiopathic male infertility and found increased urinary concentrations of 1-hydroxy pyrene, 2-hydroxyphenyrene and some PAH metabolites were associated with increased male idiopathic infertility risk. Thus, consumption of certain foods may have some risk associated with reproductive dysfunction. However, more studies are needed on this aspect.

**Tobacco smoking and chewing**

Tobacco is one of the most addictive substances. Early initiation of tobacco use by adolescents is a major public health concern. Globally, nearly 5 million people die every year from tobacco-related illnesses, with disproportionately higher mortality occurring in developing countries. It is estimated that by 2030 10 million people will die every year from tobacco use, with 70% of those deaths occurring in developing countries alone.

Several studies suggested role of tobacco smoking in deterioration of seminal quality. Reina Bouvet et al., in men with idiopathic infertility having habit of tobacco smoking demonstrated an alteration in sperm concentration and morphology with an elevation of immature forms. Lower sperm penetration assay scores and greater numbers of leukocytes in the seminal fluid were also noticed in smokers. Smokers had decreased results of hypoosmotic swelling test and increased concentration of leukocytes than non-smokers among infertile subjects whereas no differences were found in sperm concentration, percentage normal forms, different sperm defects, induced acrosomal reaction and aniline blue staining test between these two groups. Hence, it was suggested that cigarette smoking deteriorates semen quality, which could worsen fertilizing capability in infertile men. Shen et al. suggested that cigarette smoking enhance the extent of DNA damage in sperm. The extent of oxidative damage among smokers was associated with decrease in the anti-oxidant defences in the sperm of infertile males. Smokers can suffer from some degree of impotence or reduction in their sexual frequency. Cigarette smoking may also increase the risk of aneuploidy for certain chromosomes and those men may have different susceptibilities to aneuploidy in germ cells.

Study on male subjects undergoing infertility evaluations, showed a positive association of tobacco chewing and decrease in sperm quality. Tobacco chewing is more widespread in south east Asian region as compared to western countries. Among oligospermics it was observed that men with habit of tobacco chewing had reduced sperm count and motility (non-significant) than non-chewers. The percentage of men with azoospermia and oligo- asthenoteratozoospermia rose with the level of addiction. Further, experimental studies also revealed a significant increase in sperm head shape abnormality and reduction in sperm and spermatid count as well as daily sperm production after exposure to panmasala gutkha in mouse. As a consequence of panmasala treatment to both male and female mice, decline in reproductive performance and pregnancy outcome was observed. To elucidate the mechanism behind this, role of oxidative stress, sperm DNA damage was studied and the results suggested that they might play a role in panmasala-induced spermatotoxicity (unpublished).

Poor semen quality was also observed in men with a prenatal exposure to tobacco smoke. Further, tobacco smoking significantly lowered zinc levels, necessary for the stability of the sperm chromatin, in the ejaculates of smokers than in non-smokers. Zinc has a positive role in male reproduction in the form of positive correlation between seminal plasma zinc level with sperm count and α-glucosidase activity. Later Doshi et al., also showed that the mean zinc levels were lower among azoospermic as compared to oligospermic and normozoospermic subjects.

Tobacco smoking is found to alter various sex hormones. Serum E2 and prolactin (P) were increased in smokers as compared to non-smokers. In a recent study, serum levels of FSH were higher among non-smokers as compared to smokers whereas no significant differences were found for inhibin B, testosterone, SHBG, luteinizing hormone (LH) and oestradiol. However, earlier a positive dose-response relationship between smoking and
estosterone, LH and the LH/free testosterone ratios was observed. Various studies reported incompatible findings on the effects of smoking on serum hormones. There may be some effect of tobacco smoking on testosterone level as Kapoor and Jones hypothesized that the effects of smoking on estosterone levels are due to changes in plasma-binding capacity rather than a direct effect of nicotine on androgens. Smoking in patients undergoing IVF in vitro fertilization) and GIFT (gamete intra-allopian transfer) can have negative impact on treatment outcome. The data suggest the role of tobacco smoking and chewing in deteriorating semen quality or even might have effect in IVF outcome.

**Alcohol**

Humans have consumed alcoholic beverages since pre-historic times, for a variety of reasons. Alcohol is the second most addictive substance after nicotine. Alcohol is reported as direct testicular and Leydig cell toxin. It has been observed that chronic alcohol use was common among infertile men. Excessive alcohol consumption had the potential to decrease an already low percentage of sperm with normal morphology. Martini et al. also found significant reduction in seminal volume, sperm concentration, percentage of motile spermatozoa, and a significant increase of the non motile viable gametes among men with habits of alcohol and smoking. Greater numbers of leukocytes in the seminal fluid were found in alcohol users than in nonusers. But studies among healthy male volunteers with habituation of alcohol showed no significant effect on sperm nuclear size, shape, or chromatin texture and sperm concentration, motility, viability, and normal morphology. However, majority of studies reported adverse effects of alcohol on semen quality.

Alcohol use affects the hypothalamic-pituitary-gonadal (HPG) axis, a system of endocrine glands and hormones. Alcohol use is associated with low testosterone and altered levels of FSH and LH and can hence interfere with hormone production. In alcoholics, FSH, LH, and E2 levels were significantly increased, and testosterone, semen volume, sperm count, motility, and number of morphologically normal sperm were significantly decreased whereas no significant change was noted in P levels. It can be inferred that chronic alcohol consumption has a detrimental effect on male reproductive system and affect reproductive organs directly or indirectly via hormonal production and regulation.

**Stress**

Emotional and physical changes and environmental components may lead to stress. Infertility itself is the most stressful event in the lives of the person diagnosed with this condition. In general, stress affects biological systems leading to various impairments and may affect reproductive health. In many instances, stress has a subtle and less influence. Psychological job strain does not seem to affect male reproductive function. A prospective study showed small or nonexistent effect of a man's daily life psychological stress on his semen quality. Moreover, no consistent association between stress and serum concentration of LH, FSH, inhibin B, testosterone, or estradiol was found. Earlier Negro-Vilar in an overview mentioned that chronic or severe stress in animals or humans was associated with decrease sperm count, motility and morphology.

In couples suffering from involuntary childlessness, a higher frequency of male sexual disturbances expressed as erectile dysfunction, ejaculatory disorders, loss of libido and a decrease in the frequency of intercourse was observed. Allocating a mental stress caused by final exams negatively affected semen quality during stress period compared to the non-stress period. Mental stress leads to low testosterone levels due to a reduction in LH pulse frequency. The data point some adverse effect of stress on male reproductive function or as an additional risk factor for subfertility and this depend upon the types of stress.

**Heat**

Temperature influences the development of germ cells as well as reproductive cycle of living beings. Nature has kept the scrotum outside the body cavity so that the temperature of the testes remains lower than that of the body temperature. Even moderate or physiological elevation in scrotal skin temperature is associated with a substantially reduced sperm concentration, which results in a poor semen quality. Lahdetie reported that active sperm production is dependent on an environment that is 4°C lower than the normal body temperature. Wang et al., reported that elevation of testicular temperature by 1°C above the base line depresses spermatogenesis by 14% and thereby decreases sperm output. They also mentioned that exposure to high temperature results in modification of sperm morphology. The mean value of
perms with abnormal morphology rises from 30 to 60% within 6-8 months of exposure to high temperature. They explained that elevated testis hyperthermia decreases the synthesis of sperm membrane coating protein, which in turn results in the elevation of morphologically abnormal sperms in the ejaculate.

Recently, Dada et al.71,72, suggested that exposure to high temperature causes deterioration in sperm morphology and impairs motility as well as sperm production that has resulted into a deleterious effect on male fertility. Heat, either due to endogenous (such as high fevers) or exogenous stimuli, decreases sperm concentration, impairs motility, and reduces the number of morphologically normal sperm. The data on occupational health exposure and male fertility was reviewed by Thonneau et al.73. They mentioned that the use of testicular temperature induced by cryptorchidism, extreme heat in summer, body fever, tight clothing, sauna or exposure to high temperature during occupational exposure can cause impairments in spermatogenesis. The toxic effect of wet hyperthermia on semen quality may be reversible in some infertile men as observed by Shefi et al.74. Further, Zorgniotti and MacLeod75 have reported an improvement of sperm alterations in men wearing a cooling device inducing a chronic hypothermia of the testis. A weak association of heat with male fertility (OR=0.85) has also been reported.

There are few reports on reproductive health of workers occupationally exposed to high temperature. Aiga-Talmanca et al.76, mentioned a higher prevalence of pathologic sperm profile among the exposed subjects of ceramic industry compared to control. Bonde77, who studied metal arc alloyed steel welders with a moderate exposure to radiant heat but without substantial exposure to welding fumes toxicants, experienced a reversible decrease in semen quality. Sperm morphology also deteriorated during six weeks of exposure and increased after a break in the exposure. As per Kumar et al.78, welding may have had some adverse effects on sperm motility, morphology and physiologic function even though sperm concentration was in the normal range.

Kong et al79, studied the expression of cdc2 and cyclin B1 (key components of cell cycle controlling machine which are believed to play a fundamental role in gametogenesis) in normal and cryptorchid testis and observed that the abdominal temperature had no significant influence on the transcription of cdc2 and cyclin B1 in the spermatogonia and pachytene/diplotene primary spermatocytes, but it blocked the translation of them. They suggested to study the role of cold-shock proteins of spermatogenic cells at the scrotal low temperature to uncover the functions of cold shock proteins in relation to spermatogenesis, including the control mechanism of gene transcription and translation in heat induced spermatogenic block. Based on experimental studies, it was reported that elevation in abdominal temperature increases the risk of apoptosis in spermatogenic cells, but its mechanism is not clear. These data suggest the possible role of temperature on male reproductive function.

Drugs

Chronic medication can play a significant role in the pathogenesis of male reproductive health. It is known that some of the drugs/compounds may reach to the seminal plasma. There are evidence that many drugs enter the male genitourinary tract by an ion-trapping process. Lipid solubility and the degree of ionisation of the drug, which depend on the pH of plasma and seminal fluid, are important factors in this process. Major groups of drugs that may affect male sexual function include drugs of abuse, central nervous system depressants, antihypertensives, and anticholinergics, and psychotherapeutics agents. In this review, psychoactive and narcotic drugs are considered which can produce psychological dependence. Narcotic drugs exert their primary effect on the hypothalamic-pituitary axis and their secondary effects are on the gonads and sex accessory organs. Narcotics decrease gonadotropin secretion and stimulate P secretion, both of which are inhibitory to male sexual function.

The primary effect of marijuana, the most widely used psychoactive cannabis drug, is at the level of the hypothalamus, with subsequent effects on gonadotropins and testosterone and at is reported as a gonadal toxin. Infertile couples with habituation of marijuana showed greater numbers of leukocytes in the seminal fluid without any effect on sperm count, motility or percentage of oval sperm. Further, delta-9-tetrahydrocannabinol (THC) a recreational cannabis drug reduced the percentage of progressive sperm motility and acrosome reactions in vitro. In addition, the THC induces block of Gnadotropin releasing hormone (GnRH) release resulting in lowered LH and FSH and subsequently reduced testosterone production. THC appears to depress P, thyroid gland
unction, and growth hormone while elevating adrenal ortical steroids. These data suggest that THC dversely affect male reproduction both at hormonal and permatogenesis level.

A significant negative correlation was also found between the duration of khat consumption, another drug of abuse, and semen parameters. The total mean percentage of deformed spermatozoa at ultrastructure evel was approximately 65%. Deformed heads showed aberrated nuclei with immature nuclear chromatin and polyomorph intranuclear inclusions; these were associated with acrosomal defects. Persistent ytoplasmic droplets were also observed frequently. It is known that cocaine, stimulant of the central nervous system, can result in hyperactivity, restlessness, increased blood pressure, increased heart rate, euphoria etc. Bracken et al. mentioned that male population with the high prevalence of cocaine use were sub fertile with a decrease in sperm count and motility. Yelian et al. demonstrated that human spermatozoa, acutely exposed to high concentrations of cocaine decreased two notion kinematics of sperm, straight line velocity and linearity but had no significant effects on sperm motility and fertilizing capability. In another study, exposure of nales to cocaine did not decrease viability and motility but has been linked to abnormal development of their offspring as the sperm may act as a vector to transport cocaine into an ovum.

Among heroin addicts, an elevation in circulating total hyroxine, triiodothyronine and P level while depletion in serum concentrations of testosterone, FSH and cortisol were observed. Semen analyses of heroin addicts and from the dual heroin-methadone users were abnormal, whereas 45% of the methadone takers were pathological. In all cases asthenospermia was one of the abnormalities whereas 24% cases showed teratospermia and typospermia and 17% showed oligozoospermia. Such semial pathology, especially of forward motility, even in combination with normal hormone levels, may be an early indication of heroin toxicity to the male reproductive tract. The information available on various drugs of abuse especially cannabis or cocaine suggests that they have adverse effect on semen quality.

Radiation-electromagnetic radiation
Radiation can be classified as ionizing or non-ionizing radiation. It is known that ionising radiation affects both male as well as female reproduction. Non-ionizing radiation refers to any type of electromagnetic radiation i.e., near ultraviolet, visible light, infrared, microwave, radio waves, low frequency (radio-frequency) and static fields. There has been growing public concern on the effects of electromagnetic radiation (EMR) on human health including possible association with increased risk of cancer and effects on cellular DNA. A number of animal studies showed that electromagnetic waves have a wide range of damaging effects on the male reproductive system and sperm parameters. However, similar studies are limited in humans. EMR emitted by cellular phone significantly reduces human sperm motility. Use of cell phones is reported to be associated with deterioration in semen quality by decreasing the sperm count, motility, viability, and normal morphology as the duration of daily exposure to cell phones increases. Agarwal et al. concluded that radiofrequency electromagnetic waves emitted from cell phone may lead to oxidative stress in human semen based on in vitro study. Long-term EMR exposure may lead to behavioural or structural changes of the male germ cell that may be observed later in life. Still more work is needed on the use of cell phone and fertility with better study design by incorporating confounding factors associated with fertility.

Military personnels exposed to high frequency EMR through aerial and communication equipment had significant linear trends with lower ratio of boys to girls at birth and higher prevalence of involuntary childlessness. Susa et al. reported that radiofrequency fields could interact with charged intracellular macromolecular structures and could affect the mammalian reproductive system and sperm cells. In population-based studies a wide range of RF frequencies from occupational or residential exposures, no strong associations on birth defects, fertility, neuroblastoma in offspring, and reproductive hormones were found.

Lifestyle factor and IVF
Lifestyle factors may also affect the IVF outcome. Recently Klonoff-Cohen reviewed the data on the role of female and male lifestyle habits (specifically smoking, alcohol and caffeine use, and psychological stress) on the reproductive endpoints of IVF. He mentioned that there is compelling evidence that smoking has a negative influence on IVF outcomes, whereas for stress, the evidence is suggestive but insufficient due to the heterogeneity of studies. The evidence for the effects of alcohol and caffeine on IVF
outcome is inadequate. Bellver also reported that psychological stress, consumption of caffeine, alcohol and illicit drug have been implicated in a poorer IVF outcome, but evidence is inconclusive due to the scarcity and inadequate methodology.

**Conclusion**

Some "negative" lifestyle factors may be contributing to the increasing trends in male infertility problem in various parts of the world. There may not be conclusive evidence for the entire lifestyle factor discussed, but adopting healthy lifestyle may be useful at least in part in prevention of reproductive problem. The people should adopt healthy way of living in order to reduce or control the infertility problem.

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